CHARACTERISATION AND CONSERVATION OF PROMISING GENOTYPES OF ORCHIDS FROM CENTRAL WESTERN GHATS

by

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DECLARATION

I, hereby declare that this thesis entitled **"CHARACTERISATION AND CONSERVATION OF PROMISING GENOTYPES OF ORCHIDS FROM CENTRAL WESTERN GHATS"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

This is to certify that this thesis entitled **"CHARACTERISATION AND CONSERVATION OF PROMISING GENOTYPES OF ORCHIDS FROM CENTRAL WESTERN GHATS"** is a record of research work done independently by Mr. Yusuf Abbas (2014-11-151) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

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Abbreviations:

µl	: Micro litre
µg	: Micro gram
AFLP	: Amplified Fragment Length Polymorphism
AMOVA	: Analysis of Molecular Variance
BSI	: Botanical Survey of India
Bp	: Base pair
BWS	: Brahmagiri Wildlife Sanctuary, Kodagu, Karnataka
CBAFS	: Coffee Based Agro Forestry System
CITES	: Convention of International Trade in Endangered Species of Fauna and Flora
cm	: Centimeter
CoA	: College of Agriculture
CSIR	: Council of Scientific & Industrial Research, New Delhi
CTAB	: Cetyl Trimethyl Ammonium Bromide
DNA	: Deoxy Ribo Nucleic Acid
dNTPs	: Deoxy Ribose Nucleoside Triphosphates
EDTA	: Ethylene Diamine Tetra Acetic acid
FAA	: Formalin Acetic Acid
HCl	: Hydrochloric Acid
IABG	: International Association of Botanic Gardens
ICAR	: Indian Council of Agricultural Research
IIHR	: Indian Institute of Horticultural Research, Bengaluru.
ISSR	: Inter Simple Sequence Repeats
IUCN	: International Union for Conservation of Nature
Kb	: kilo base
Kg	: kilo gram
Km	: kilo meter
KOH	: potassium hydroxide
LAS	: Leica Application Suite
l	: litre
mg	: milli gram
mM	: milli Molar

MSL : Mean Sea Level

NBG : National Botanic Gardens, Dublin, Ireland.

NBRI : CSIR-National Botanical Research Institute, Lucknow.

NPK : Nitrogen, Phosphorous, Potassium

NRCO : National Research Centre for Orchids, Sikkim.

NTSYS : Numerical Taxonomy and Multivariant Analysis System Program Package

OD : Optical Density

ORP : Orchid Plant

PCR : Polymerase Chain Reaction

PDK : Padannakkad

pg : Pico gram

PIC : Polymorphic Information Content

PPM : Parts Per Million

PVP : Polyvinylpyrrolidone

PWS : Pushpagiri Wildlife Sanctuary, Kodagu, Karnataka

RAPD : Random Amplified Polymorphic DNA

RARS : Regional Agricultural Research Station, Pilicode

RFLP : Restriction Fragment Length Polymorphism

RGNP : Rajiv Gandhi National Park, Nagarhole, Karnataka

RNA : Ribonucleic Acid

RPM : Rotation per Minutes

SG/F : Sacred Grooves/Forest

SRL : Sisco Research Laboratories

SSR : Single Sequence Repeats

TAE : Tris-Acetate-EDTA

Taq : *Thermus aquaticus*

TBE : Tris Borate EDTA

TWS : Talakavery Wildlife Sanctuary, Kodagu, Karnataka

UPGMA : Unweighted Pair Group Method with Arithmetic Mean

UV : Ultra Violet

Introduction

1. INTRODUCTION

Orchids, with their most beautiful flower in nature, comprise of a unique group of plants distributed in every ecological situation and occupying a wide range of habitats (Kull *et al.*, 2006; Singh *et al.*, 2007) except for the frozen continent of Antarctica. Taxonomically orchids belong to the family Orchidaceae, one of the largest families of flowering plants and the most highly evolved family among monocotyledons (Cozzolino and Widmer, 2005). They exhibit highly specialized morphological, structural, and physiological characteristics. The family contains 17,000 known wild species in 750 genera in the world and the hybrids among these are around 80,000 (Dressler, 1990; Rao, 1998). However, more recent estimates suggest their number at 24,500 (Dressier, 2005) and 26,049 (World Check list of Selected Plant families: Kew, 2009). They are primarily distributed in the tropical and subtropical climates due to prevalence of dense vegetation and high humidity, as these two factors are necessary for their growth and development in nature (Vij and Pathak, 2001). The various climatic factors and diverse ecological niches of India provide an excellent abode for orchids and serve important centre of biodiversity.

India has one of the richest orchid habitats with more than 1,200 species. It is estimated that orchids form about 9 percent of flora found in India with Himalayas as their main home. They are found mostly in the North Eastern region and several are scattered in the Eastern and Western Ghats (Bhanwra *et al.*, 2006). There are about 352 orchid species which are endemic to India (Singh, 2001). The orchid species are distributed in different regions of India namely Western Ghats, North-Eastern India, and North-Western Himalayas of which the Western Ghats harbour 275 species (Rao, 1998). Out of 275species, Rao (1998) has described 65 species of orchids belonging to Kodagu region of Karnataka which is situated in the eastern slope of Western Ghats region. Brahmagiri Wildlife Sanctuary and Pushpagiri Wildlife Sanctuary are known as treasure troves of orchids in Kodagu. The rain forests are also home to some rare species of genus *Aerides, Bulbophyllum,* (*B. acutiflorum, B. mysorense and B. elangata*), *Dendrobium,*

Rhyncostylis (foxtail orchid *R retusa*) etc. *Coelogyne sp.* is found in the high altitudinal shola forest range. Muktesh Kumar and Sasidharan (1986) listed 67 genera with 190 species to occur in Kerala of which 85 species are endemic to Western Ghats and of them 15 are restricted to Kerala. Sathish Kumar (1986)

In orchids nearly 250 species are threatened of survival, while a few others have probably been vanished from their Indian habitats. Due to habit specificity, most of the orchids are susceptible to habitat fragmentation and deterioration. At present orchids are considered to be at a high risk of extinction and hence most of the orchids are included in the conservation lists (IUCN/SSC Orchid Specialist Group, 1996).

The world consumption of orchids was valued for more than 500 million U.S dollars in 2000 (Wang, 2004). Countries like Netherlands, Germany, China, Japan, Taiwan, Thailand and U.S.A are involved in large scale orchid production (Griesbach, 2000). Recently, India has started producing orchids for export.

The floral characteristics of orchids cover an exceptionally wide range of different sizes, shapes, forms, and coloration (Dressler, 1993). Orchid flowers have a longer shelf-life and as a result they are marketed globally as cut flowers, for floral arrangements and bouquets, as potted flowering plants and as bedding or aerial plants (Yadav and Basalc, 1999; Lopez and Runkle, 2005; Attri *et al.*, 2008). Due to the floral diversity and long shelf life of their aesthetically beautiful flowers, these plants have often been mentioned as 'Gems of Nature' in cut-flower industry. Orchid based floriculture has a flourishing trade in several countries including USA, Malaysia, Thailand, Singapore, Japan and Australia.

A large number of Indian orchid species under the genera *Aerides, Arachnis, Aranthera, Arundina, Cattleya, Coelogyne, Cymbidium, Dendrobium, Paphiopedilum, Papilionanthe, Renanthera, Vanda* etc. have ornamental value and are extensively used for the production of a number of hybrids (Kumar *et al*, 2001). More than 1,00,000 orchid hybrids have been produced commercially and hundreds of new hybrids being added every year (Vij and Gupta, 1997).

Apart from ornamental use, the orchids have therapeutic importance also. As orchids are rich in phytochemicals, these are extensively used in local medicines (Toh, 1994; Hussein and Rahman, 2003; Rao, 2004; Nayak *et al.*, 2005; Jalal *et al.*, 2008a) to cure a number of ailments including dysentery (*Cymbidium canaliculatum, Habenaria conopsea*), haemorrhage (*Cymbidium giganteum, Dendrobium loddigesii*), worm infection (*Epidendrum bifidum, Dendrobium discolor*), and pain (*Dendrobium teretifolium*) (Lawler and Slaytor, 1970; Handa, 1986; Chen, 1994; Kong, 2003). A phytochemical known as moscatilin, derived from *Dendrobium loddigesii* has been reported to have some anti-cancer activity for stomach and lung cancer cell line (Ho, 2003).

In the Ayurvedic system of medicine, several orchid species including *Malaxis acuminata* (Rishbhak), *Malaxis muscifera* (Jeevak), *Habenaria intermedia* (Ridhi), and *Habenaria edgeworthii* (Vridhi) are used in a number of rejuvenating formulations such as 'Chyavanprash' (Dey, 1982). Many Indian orchids have been reported to cure rheumatism (*Acampe papillosa, Rhynchostylis retusa*), malignancy (*Goodyera pubescens, Vanda testacea*), respiratory ailments (*Coelogyne henryi, Dactylorhiza hatagirea*), orthopaedic complaints (*Cymbidium aloifolium*), cardiac problems (*Eulophia campestris,*), dysentery (*Liparis rostrata, Satyrium nepalense*) and nervous disorders (*Cymbidium elegans, Dendrobium nobile*) (Kumar *et al.*, 2000; Singh, 2001; Rao, 2004; Nayak *et al.*, 2005; Dash *et al*, 2008). Antimicrobial properties of a variety of orchids have also been tested (Ghanaksh and Kaushik, 1999; Dayamma and Rampal, 2002; Lal *et al.*, 2004).

Orchids are also used as source of food, glues, gums, perfumes, essences, narcotics, and even as poisons (Lawler, 1984). Vanillin, aromatic oil exuded from the green pods of *Vanilla planifolia* is the most famous commercial product from orchids.

Though sexually propagated in nature, orchids produce non-endospermic seeds which are microscopic and with highly reduced embryos which require mycorrhizal association for their germination. The high rate of cross pollination

and the long distance seed dispersal of orchids by wind (Arditti and Ghani, 2000) are responsible for high rates of gene flow between population. This results in higher level of genetic variation within populations and low degree of genetic differentiation among populations (Cozzolino and Widmer, 2005).

The habitat destruction and uncontrolled collection have resulted in reduction in number of orchid species India (Pradhan, 1985) and this is applicable to the Western Ghats region also. Therefore, documenting of regional data regarding the orchid diversity with comprehensive morphological and ecological notes in relation to their habitat is important. The understanding of the level of genetic variation within and among populations is needed for conservation and sustainable utilization. Population genetic studies are essential for conservation program and restoration of threatened populations. Evaluation of genetic diversity plays an important role for their conservation and management (Hamrick and Godt, 1996). Comparative population studies using molecular and biochemical markers are needed to collect information on the level and pattern of genetic diversity of wild orchids, which is the initial step towards their conservation (Moller and Spoor, 1993; Geburek, 1997). To obtain consistent information on the existing genetic diversity, a number of reliable and widely used markers have been developed in orchids. RAPD and ISSR analysis are popular methods for estimating genetic diversity in plant populations.

Hence the present study on 'Characterisation and conservation of promising genotypes of orchids from Central Western Ghats' has been initiated by conducting a survey in the Central Western Ghats region to document the various species available and to rescue, conserve and characterize the different orchid genera using morphological and DNA fingerprinting techniques like RAPD. This will contribute to the better understanding of the genetic profile that can be used to develop strategies for their conservation and sustainable utilization in future. This will also form a starting point for future research on population and evolutionary genetics of these species.

Hence, the present investigation was carried out with the following objectives:

- To rescue and characterize orchids from Central Western Ghats using morphological and molecular markers.
- To conserve under *Ex-situ* condition as well as in an orchidarium at College of Agriculture, Padannakkad.
- To identify promising genotypes from collected sample.

Review of Literature

2. REVIEW OF LITERATURE

Orchids are the unique group of flowering plants occurring in abundance in humid tropics and also in temperate areas of India. The orchids belong to the *Orchidaceae* family, which is one of the most ecologically and morphologically diverse families of flowering plants in the world. The family comprise of about 850 genera and 30,000-35,000 species (Dressler, 1993, Lucksom, 2007). Based on the different habits they grow, orchids are classified into saprophytes (orchids which grow on dead and decayed matters), terrestrials (orchids which grow on ground) and epiphytes (orchids which grow on trees and other supports). Among the orchid population, 70% of them belong to the epiphytic class (Graveendeel *et al.*, 2004).

Orchids which are either epiphytes or terrestrial are infested by the mycorrhizae to absorb nutrients from the soil. The aerial root system have the capacity to absorb moisture from the atmosphere. Stem is swollen and fleshy at the bottom and have simple leaves in pair or in cluster with variegated colour and patterns. Orchids show high variations in flower shape, size, odour etc. and they have symmetrical or asymmetrical floral parts. Orchids have mechanism to pollinate the flower by mimicking insects, spider, birds etc and the pollinator gets tricked by mimicry and land on the flowers.

2.1 Geographical distribution:

Orchids are cosmopolitan with a wide distribution all over the world. They can be found in every habitat except in the coldest and driest regions (Seidenfaden and Wood, 1992). Generally the Orchidaceae family has been diverse in wet conditions such as the tropical regions (Batygina *et al.*, 2003) and has wide range of habitats as they can grow from sea level up to 4200 m (Hodgson *et al.*, 1991). This family is also the largest flowering plant families including 10% of the higher plants.

In India, orchids are found distributed in the different parts ranging from different altitude, rainfall, and temperatures. They are found in altitude above

5000 meters above mean sea level (MSL). Orchids are also distributed in areas where rainfall ranges from 60 to 1100 cm/ year. Based on the distribution of orchids in India, the orchid growing zones are classified into i) tropical, ii) sub tropical, iii) temperate, iv) sub-temperate, v) alpine and vi) sub alpine zones (Bose *et.al.*, 1999). In India the orchids are mainly distributed in the Eastern Himalayas, Western and South Indian hills. The Western Ghats of South India and the North-Eastern Region of North India are the hotspots of orchids with the richest biodiversity (Singh *et al.*, 2009). Western Ghats in the South of India is the home to about 275 species of orchids (Rao, 1998). There are more than 65 species of orchids reported to be present in the Kodagu region of Karnataka, which is situated in the eastern slope of Western Ghats. Brahmagiri wildlife Sanctuary and Pushpagiri wildlife sanctuary are known as "treasure troves" of orchids which are located in the Kodagu district of Karnataka state.

With valid and well acceptable modern taxonomic classification based on morphological, cytological, embryological, phytochemical, ecological and molecular parameters, identifying a species has more accuracy. Although majority of the orchids are epiphytes, their habit is greatly influenced by different environmental and ecological factors; and these are variously adapted to terrestrial, lithophytic, and subterranean mode. The qualitative abundance of orchids in mountainous regions in India has been influenced by physicochemical and biotic factors (Sathish Kumar and Manilal, 1994; Vij, 1995). Rao and Khasim (1986) have assessed the taxonomic significance of anatomical features and indicated their relationship with the ecological adaptability.

2.2 Botanical description

Orchids are monocots, perennial herbs with simple leaves and parallel veins. Orchids are well known for the beauty and rich diversity of their flower structures (Bandisch, 1998). Some orchid species possess a single flower, whereas the majority of the species have inflorescences with multiple flowers arranged around a stalk.

2.2.1 Stem

Orchids are perennial herbs and lack any permanent woody structure. Orchids with sympodial growth have a specialized lateral growth pattern in which the terminal bud dies. The growth continues by development of new shoots sprouting from the base of the stem of sympodial epiphytes, or in some species essentially the entire stem may be thickened to form what is called a pseudo bulb. The pseudo bulbs are storage organs derived from the part of a stem between two leaf nodes. The rhizome might fork into two and with aging the pseudo bulb sheds its leaves and becomes dormant. At this stage it is often called a back bulb. A pseudo bulb then takes over, exploiting the last reserves accumulated in the back bulb, which eventually dies off. In warm and humid climates, many terrestrial orchids do not need pseudo bulbs.

Monopodial orchids grow upward from a single point. They add leaves to the apex each year and the stem grows longer accordingly. Orchids with monopodial growth often produce many aerial roots that often hang down in long drapes and have green chlorophyll. These orchids are devoid of rhizome or pseudo bulbs.

2.2.2 Leaves

Orchid leaves are green in colour and monocotyledonous in nature. Leaves are long, plicate or short, elliptic oblong or linear ovate. In epiphytic species leaves are thick and coriaceous. In species like *Vanda*, the leaves act as a storage organ, whereas the leaves are laterally compressed and succulent in *Oberonia* and *Podochilus* (Bose and Bhattacharjee, 1980; Bose *et al.*, 1999)

2.2.3 Inflorescences

The origin of inflorescence is specific to each genus and species. They are either terminal or lateral in terrestrial orchids, while in the epiphytic forms the inflorescence is lateral type and usually develops from the leafy shoot.

2.2.3.1 *Flower*

Orchid flowers are zygomorphic in nature (Bose, *et al.*, 1999) and well known for their diversity (Mondrago'n-palomino and Theisen, 2008) in flower colours and shapes. The shades of the flower are white, yellow, green, purple, or a combination of all these colours.

The orchid flower typically consists of an outer whorl of three sepals, an inner whorl of three petals, and a single large column (the gynostemium, composed of the male stamens attached to the female pistil) in the center. The sepals are the protective cover of the flower bud. The two lateral petals flank the greatly enlarged flamboyant bottom petal (lip or labellum) which is usually highly modified to attract the pollinators (Rudall and Bateman, 2002). The lips are generally differently coloured with lot of ornamentations, such as crests, tails, warts, hairs, and teeth. The orchid's reproductive organs are combined into a single column known as gynostemium, which is the primary identification feature of orchids (Dressler, 1993; Rudall and Bateman, 2002). The top part of the column is provided with small packets of pollens which are generally termed as pollinia. The morphological characteristic of pollen grains and spores are important criteria in consideration of the taxonomy and inter-relationships of plants at various taxonomic levels.

Below the anther is the stigma, usually a shallow and sticky cavity in which the pollen is placed for fertilization. There is a small growth, called the *rostellum* which acts as a protective barrier to prevent self pollination.

In the bud stage, the lip is the uppermost petal which is then twisted around 180° when the flower opens by a process called resupination. Many tropical species of orchid rely on a single species of euglossine bee to pollinate them. Some orchid species have established pollinator relationships with flies, moths, butterflies, hummingbirds and with even bats (Jersáková *et al.*, 2006).

2.2.4 Seed

Orchid seeds are very small dust like and contain little reserve food. These are generally less than 2mm in length and 1 mm in width and dispersed easily by wind (Brundrett et al., 2001). The orchids seeds have no stored nutrients for growth and cannot survive without a mycorrhizal interaction. The seeds contain little more than a rudimentary embryo and no endosperm (Arditti, 1992). The orchid seed consists of a testa surrounding a tiny embryo in the globular stage with no defined cellular organization suggestive of embryonic tissue found in other angiosperms (Arditti, 1992). Upon germination, the embryo swells to form a protocorm that will develop rhizoids. After a few days (or several weeks) the protocorm develops a shoot apex with leaf primordium and a root will form. The chalazal end of the embryo develops into the shoot apex and roots arise from other parts (Arditti, 1979). During the early stages of growth prior to shoot apex formation, cell division takes place in the chalazal end of the embryo while cells enlarge but show little or no division at the micropylar end. Most of orchid seeds cannot germinate or start germinating but will not grow, unless they are infected with mycorrhizal fungi which supplies young plants with all the sugar and nutrients (Brundrett *et al.,* 1996; 2003). The study of the morphological characteristics of the seeds can be also useful in various taxonomic and phylogenetic aspects of orchids.

2.3 Germination system in Orchids

There are two method of propagation and conservation of orchids namely asymbiotic method, symbiotic method.

2.3.1 Asymbiotic seed germination

Asymbiotic seed germination has become the favoured method of orchid propagation and production. In tropical epiphyte orchids, the germination is usually accomplished using synthetic medium which are well standardized. However, the germination method in vitro is unsuccessful in the case of terrestrial orchids. (Arditti *et al.,* 1981; 1990; Smith and Read, 2008)

2.3.2 Symbiotic seed germination

In symbiotic seed germination the seeds are infected with the specific fungi and start to grow. The advantage of this approach is that, media used is simple containing oats powder and some yeast extract and the resulting l plant are stronger and more resistant to pathogens such as fungal disease than asymbiotic plants (Brundrett et al., 2001).

2.4 Growth habits

Orchids have varying growth habit to adjust with different environmental conditions in which they grow. Based on the habits which they grow, orchids are classified into terrestrial, epiphytic, and saprophytic forms (Jonathan and Raju, 2005).

2.4.1 Terrestrial orchid

Terrestrial orchid are those orchid plants which can grow in the soil and they make approximately 12 percent of the total number of species of orchids (Brundrett *et al.,* 2001). There are mainly two types of terrestrial orchids, those that have tubers and pseudo bulbs. These are perennial in habit and dies back during cold or dry spell and grows rapidly and flower during spring (Smith and Read, 2008; Brundrett *et al.,* 2001). *Habenaria caranjensis, Habenaria elwesii and Malaxis intermedia* are a few examples of terrestrial orchids. The important feature is that these orchids have a symbiotic relationship with specific fungi. It was also noted that when these wild terrestrial orchids are transplanted from its natural habitats, it will die or become unhealthy because of the new environmental conditions which lacks the fungus. This underlines the influence of fungi on the orchids for their better development and growth (Rasmussen; 1995, Smith and Read, 2008).

2.4.2 Epiphytic orchids

Epiphytic orchids are those orchid plants which entirely depend on trees for the structural and nutritional support. They are not parasitic and do not feed on

the host plants (Dematte and Dematte, 1996, Jonathan and Raju, 2005). *Dendrobium aqueum, Dendrobium nanum,* and *Eria mysorensis* are a few examples for epiphytic orchids. These orchids obtain nutrients from rain and debris which accumulated on the barks of host plants. Epiphytic orchids have adapted to have aerial roots and do not have the advantage of absorption of the water from the soil. In orchids, the water is absorbed by spongy structures called velamen tissue which is formed by dead cell layers in roots (Bomba, 1975; Batchelor, 1981; Dematte and Dematte 1996) The epiphytic orchids are common in North-Eastern India which grows up to an elevation of 2000m from MSL. Most of the *Paphiopedilum* (lady's slipper) species are restricted to North Eastern Himalayas except for *P. druryi* which was reported from Kerala but now is almost extinct from its original habitat (De and Medhi, 2013).

2.4.3 Saprophytes

Saprophytic orchids are very small in number and they have peculiar characteristic possessing mycorrhizal fungus in their root for the supply of nutrients. These orchids do not have the chlorophyll and they have the coralloid roots, i.e., roots with reduced size which are seen underground and emerge out of the ground only during the flowering stage. *Didymoplexiopsis khiriwongensis, Stereosandra javanica* and *Epipogium roseum* are a few examples of saprophytic class of orchids.

2.5 Orchids in India

Orchids form 9 percent of flora and are the largest family among higher plants in India. It is estimated that about 1,300 species (140 genera) of orchids are found in India with Himalayas as their main home and others scattered in Eastern and Western Ghats.

The orchid species are distributed in different regions of India. It is estimated that Western Ghats is the home for approximately 300 species, regions in North-Eastern India have around 200 species, and North-Western Himalayas with the maximum of 800 species.

Northeastern India has varied climatic conditions and contains largest group of temperate and sub-tropical orchids. In about 132 wild genera nearly over 71 genera are endemic Orchids, and believed to have evolved in this region (Kumaria and Tandon 2007). Of about 1331 species of orchids belonging to 186 genera reported from India, (De, 2013) Northeast India sustains the highest number of about 850 species. As many as 34 species of orchids from North East India are listed among the threatened plants of India (Nayar and Sastry 1987, 1988, 1990) and 85 species are endemic to this region (Das and Deori 1983). Out of the eight orchid habitat regions in India, the two most important areas namely; the Eastern Himalayas and the Northeastern Region fall within the political boundaries of North Eastern Region. They are unique to the region and are not found anywhere in the world.

Terrestrial orchids are located in humus rich moist earth under tree shades in North Western India. Epiphytic orchids are common in North-Eastern India which grows up to an elevation of 2000m MSL. Orchid species with high ornamental values originated from this region are *Aerides multiflorum, Aerides odoratum, Arundina graminifolia, Arachnis,Bulbophyllum, Calanthe masuca, Coelogyne elata, Coelogyne flavida, Coelogyne corymbosa;Cymbidium aloifolium, ;Cymbidium. lowianum, ;Cymbidium devonianum, ;Cymbidium hookerianum, ;Cymbidium lancifolium,Dendrobium aphyllum, Dendrobium nobile, Dendrobium chrysanthum, Dendrobium farmeri, Dendrobium chrysanthum, Dendrobium densiflorum, Dendrobium moschatum, Dendrobium. fimbriatum, Dendrobium jenkinsii, Paphiopedilum venustum, Paphiopedilum spicerianum, Paphiopedilum hirsutissimum, Paphiopedilum insigne, Phaius wallichii, Pleione praecox, Renantheraimschootiana, Rhyncostylis retusa, Thunia alba, Vanda cristata, Vanda coerulea and Vanda coerulescens* (Singh 1990).

2.6 Cultivation Practices

2.6.1 Orchidarium

The controlled green houses where the orchids are cultivated or conserved are known as orchidarium. There are two types of green houses used in the cultivation of orchids, uncooled type, and controlled type. Tropical warm types are mostly grown in uncooled green houses which protect the plant from the direct sunlight and also reduces the inside temperature and increase humidity favoring the luxuriant growth and flowering of orchids (Bose *et al.*, 1999). In the controlled conditions, temperature, light and humidity are maintained which favour the environmental conditions for growing high valued orchids.

2.6.2 Containers Used For Growing Orchids

Varieties with different shapes and sizes of containers are used for growing orchids. The most commonly used ones are the clay pots, tree ferns log and galvanized mesh and the plastic pots. The pots are provided with pores or cuts to facilitate the aeration and proper drainage of water. Mostly the plastic containers are preferred, as they have less weight, easy transportation, free from deposition of salts and algae on the outer side and longer moister retaining capacity than clay pots (Black, 2003).

2.6.3 Potting Mixture

Suitable growing media for orchid is necessary for the growth and development and proper flowering. The media should support the plant, supply water and nutrients to the roots, provide good drainage and aeration. In the past years orchids have been grown in different potting media (Tan and Lee, 2001; Lauzer *et al.*, 2007).

Charcoal and the broken tiles have been used in 2:1 ratio as reported in *Vanda* (Seeni and Latha., 2001). Temjensangba and Deb (2005) used mixture of coal pieces and brick pieces, coconut husk and decayed powder (1:1:1:1) with layer of moss to achieve 60% survival rate of the hardened plants. Rooted plants

were cultured on a pre hardening media containing Paclobutrozol (0.25mg/l) and activated charcoal (1.5mg/l) for two months. After the hardening, the plants were transferred to pots containing the mixture of sterilized coco peat and tree ferns (1:1). Chen *et al*., (2005) successfully utilized mixture of perlite, sand, charcoal and soil (3:2:2:3) for hardening *Cymbidium fabereri* plants. Regenerated plants of *Oncidium* Spp (Dancing dolls) were grown in the green house after transferring to perforated plastic pots containing mixture of charcoal and bricks pieces (1:1) (Kalimuthu *et al.,* 2007). It has observed that 80 percent of survival rates in *Dendrobium chrysotoxum* by use of dry coconut husk and charcoal and pieces of bricks in (1:1:1) ratio (Roy *et al*., 2007).

2.7 Nutrient Requirement

The supply of nutrients in liquid form improves the growth and flowering of orchids. In the natural habitats, orchids get their nutrients from the dead and decay organic matter and soil on which they are grown and from the atmosphere through rain (Bose *et al.,* 1999). During cultivation, all the nutrients are required to be supplied to the orchids regularly for their growth and development and flowering (Black, 2003). Several fertilizer solutions have been recommended by various workers.

The studies conducted by (Kumarai and Tandon, 1994) reported that in *Dendrobium fumbriatum* var *oculatum* plantlets, feeding with MS liquid medium was found to be beneficial for hardening. Similarly use of MS medium (10x) was recommended in *Arachinis labrosa* (Temjensangba and Deb, 2005), *Cymbidium devaniiam* (Das *et. al*., 2007). Seeni and Latha (2000) used foliar spray of Vijay complex (N: P: K in 17:17:17, Madras fertilizer co, Madras) at weekly intervals in case of blue *Vanda* Park *et al.* (2002) used Hyponex solution 6.5:4.5:19 N: P: K at 15 days intervals in case of *Phalenopsis.* A mixture of two commercial fertilizers i.e. DAP and NPK mixture (20/10/10) was used at weekly intervals for hardening of *Vanda coerulea* (Malabadi *et al*., 2004)

2.8 Irrigation

Quality and quantity of irrigation water is very vital for the survival of the plants. It is difficult to prescribe a schedule of watering for the orchids since the number of watering is important to the survival of the plant depending on the climatic condition and the type of the media used (Black, 2003). For most of the orchids watering should be done only when the medium is completely dry. However in case of *Paphilopedium* and *Cymbidium* species, water around the roots is required at all times. Over watering should be avoided for the wild orchid species such as *Vanda* which requires minimal supply of the water usually absorbed through the velamen roots (Bose *et al.,* 1999). Over watering blocks the air passages in the roots (Abraham and Vatsala, 1981). Wrinkling of pseudo bulbs and yellowing of leaves are the initial signs of over watering. At this point watering should be stopped immediately and confined to aerial spraying (Chen *et al*., 2005).

2.9 DIVERSITY STUDIES

Knowledge on genetic diversity forms a base for conservation (Geburek, 1997). In the past, population genetic studies have been carried out to understand the loss of genetic diversity and also to restore the threatened populations (Hamrick and Godt, 1996). Loss of the genetic variation is a major problem in the conservation of the particular species of orchids, which can prevent a species from the natural selection and limit its evolutionary potential (Qamaruz-zaman *et al*., 1998).

Orchid seeds are very small and easily get transported to long distances by wind (Arditti and Ghani, 2000). This particular characteristic is responsible for the high rate of gene flow which results in higher level of genetic variation within the population (Cozzolino and Wildmer, 2005). An understanding of genetic variation level within and among population is essential for developing appropriate conservation and sustainable utilization methods (Xiaohong *et al*., 2007). Very little is known about the genetic diversity within the natural population despite the

tremendous diversity within the family Orchidaceae (Xiaohong *et.al.*, 2007; Wang *et al.*, 2008).

2.9.1 Molecular markers for diversity analysis

In recent years the fast growing field of molecular biology has provided tools suitable for rapid and detailed genetic analysis of higher organisms. The most fundamental of these tools are molecular markers for detecting differences in the genetic information carried by two or more individuals. Molecular markers are found to be versatile tools in taxonomy, physiology, embryology, genetic engineering, etc. The discovery of PCR has brought about a new class of DNA markers and they are constantly being modified to enhance their utility and to bring about automation in the process of genome analysis.

Molecular markers have proved that they are valuable technique in the characterization and evaluation of the genetic diversity of various plants including orchids (Powell *et al.*, 1996; Russell *et al.*, 1997). Several molecular markers which include RAPD, AFLP, RFLP, and SSR have been used to study the diversity and genetic similarity of various orchid species. The RAPD technique, AFLP, RFLP, ISSR and SSR are frequently used to study diversity of the orchids (Chattopadhyay *et al.*, 2012; Sardaro *et al.*, 2012; Chang *et al.*, 2000; Schlueter *et al.*, 2007; Pillon *et al.*, 2007; Boonsrangsom *et al.*, 2008; Verma *et al.*, 2009; Jacquemyn *et al.*, 2009). In orchids, a number of reliable markers have been developed to obtain more consistent information on the existing genetic diversity (Cambell *et al.*, 2002).

The DNA markers offer several advantages over traditional phenotypic markers and have very rapidly complemented the classical strategies. This facilitated the development of marker-based gene tags, map-based cloning of agronomically important genes, variability studies, phylogenetic analysis, synteny mapping, marker-assisted selection of desirable genotypes, etc. This gave new dimension to concerted efforts of breeding and marker-assisted selection to reduce the time span of developing new and better varieties. Information from DNA

markers serves many divergent purposes like forensic science, paternity testing, identifying the genes responsible for diseases and inferring evolutionary relationships, the most wide spread being in construction of genetic maps (Patterson *et al.*, 1991).

Molecular markers could be used to trace linkage with traits of importance, especially multigenic or quantitative traits that are difficult to deal with when relying on phenotypic assay alone. This approach permits the breeder to make early decision about his selections while examining fewer plants. Molecular markers have been used to develop linkage maps for many important crop species with concentrated efforts on cereals (Helentjaris, 1987).

DNA based markers effectively replaced enzyme markers in germplasm identification and characterization and in gene tagging. Owing to its plasticity, ubiquity and stability DNA is the ideal molecule for such analysis (Caetano-Annoles *et al.*, 1991). Genetic polymorphism is classically defined as the simultaneous occurrence of a trait in the same population of two or more discontinuous variants or genotypes. Polymorphism in the nucleotide sequence is sufficient for it to function as a molecular marker. Although DNA sequencing is a straight forward approach for identifying variations at a locus, it is expensive and laborious. A wide variety of techniques have, therefore, been developed in the past few years for visualizing DNA sequence polymorphism. Various types of molecular markers are utilized to evaluate DNA polymorphism and are generally classified as hybridization-based markers and polymerase chain reaction (PCR)-based markers. But the most suitable marker systems for a particular purpose should be selected based on the information content and multiplex ratio.

Properties desirable for ideal DNA markers are highly polymorphic nature, codominant inheritance (determination of homozygous and heterozygous states of diploid organisms), frequent occurrence in genome, selective neutral behaviour (the DNA sequences of any organism are neutral to environmental conditions or management practices), easy access (availability), easy and fast

assay, high reproducibility and easy exchange of data between laboratories. It is extremely difficult to find a molecular marker which would meet all the above criteria. Marker system can be identified that would fulfill at least a few of the above characteristics depending on the type of study to be undertaken (Weising *et al.*, 1995).

2.9.2 RAPD Markers

The genetic variations between organisms are mainly studied using various molecular markers such as RAPD, AFLP, RFLP and Micro Satellites among these molecular marker RAPD is widely used to asses intra specific variation at the nuclear level. The RAPD uses a single arbitrary primer in the PCR reaction which provides a quick and efficient screening for the DNA sequence based polymorphism at a very large number of loci (Williams *et al.,* 1990). The main advantage of RAPD is that it does not require any sequence information of DNA. Reproducible RAPD bands can be formed by careful selection of primers, optimisation condition of PCR target spp and replication to ensure that only reproducable bands are scored (Kumari and Thakur, 2014). RAPD profiling has been widely used for various purposes which includes identification and classification of accessions (Fukuoka *et al.,* 1992), identification of breeds (Qian *et al.,* 1996), and genetic diversity analysis of various plant crops.

The RAPD technique has many advantages such as simplicity and rapidity of the analysis, low cost, availability of a large no of primers and the requirement of the small amount of the DNA for the analysis (Williams *et al.*, 1990; Huff *et al.*, 1993; Ge *et al.*, 1999; Nybom and Bartish, 2000). RAPD technique is more suited to orchids since very little is known about the genetic diversity within the natural population (Xiaohong *et al.*, 2007). The identification and mapping of the DNA polymorphism using RAPD technique shows species specific and genus specific traits, speciation, morphological evolution and molecular stage in the plants (Benner *et al.*, 1995)

2.9.3. RAPD for orchid diversity analysis

Lim *et al.*, (1999) confirmed that strap and terete leaved *Vanda* a is phylogenetically distinct based on the RAPD analysis and also recommended RAPD analysis for the determination of the genetic background of the plant used in the hybridization programme. Wong and Sn (1999) studied *Godyera procera*, a terrestrial orchid, using RAPD and found that the diversity varied greatly both at species and at the population level. Wallace (2002) used RAPD loci to assess the potential effects of the fragmentation and reduced population size on the future viability of the *Plantanthera leucophaea*, a threatened species. Li *et al.*, (2002) conducted a preliminary analysis of the level and apportionment of the genetic diversity in *Paphilopedium micranthum* using the RAPD marker analysis.

(Besse *et al.*, (2004) detected low level of the genetic diversity in *Vanilla planifolia* in area such as Reunion Island and (Polynesia) Pacific Ocean. Goh *et al.*, (2005) demonstrated that the RAPD markers are useful tool for the identification of *Phalenopsis* orchids up to the specific and sub generic levels. Li and Ge (2006) investigated the level and apportionment of the genetic diversity of the species using RAPD technique in *Changnienia amoena,* endemic to china. Based on there result they proposed conservation managements for this endangered species including habitat protection along with the protection of their pollinators, artificial pollination as well as *ex situ* conservation.

Low level of the diversity at species and population level between glacial and UN glaciated sites of *Cypripedium reginae* was reported based on the RAPD (Kennedy and Walker, 2007). No difference in RAPD banding pattern genetic in wild and cultivated group of *Vanilla planifolia* was observed by Schluter *et al.*, (2007). Minoo *et al.*, (2008) discriminated different species of the *Vanilla planifolia* and its related species using RAPD analysis that were differentiated by the presence or the absence of the leaves and flower colour.

Genetic diversity and population structure in the Brazilian *Cattleya labiata* were studied by Pinheiro *et al.*, (2012) using RAPD and ISSR markers. The data

generated from the study using 12 ISSR and RAPD primers were used to determine the genetic variability of the species. The study reported a total of 130 individuals of which 117 belonging to *Cattleya labiata* and 13 from 10 other species in the same genus. The marker data indicated that *Cattleya labiata* has a high level of polymorphism and five reconstructed populations were identified by the programme, 'Structure'. Other *Cattleya* species also showed no relationship with any *C. labiata* accessions. The genetic characterization of *Cattleya* from northeast Brazil contributes to knowledge of the genetic structure of the species and can be used to define strategies for conservation and breeding programmes.

Lim *et al.*, (1999) reported that the RAPD method can be used to indicate the genetic closeness of orchid species and hybrids quickly and efficiently. The study also revealed the possibilities to predict the outcome of a cross based on genotypic information. They reported the genetic distances of orchids in the genus *Vanda* and another related genus, *Ascocentrum*, using RAPD marker. The study also suggested the suitability of RAPD markers to differentiate between the two groups within *Vanda* genus and to study the relationship between *Vanda* and *Ascocentrum*.

Genetic analysis for identification, genomic template stability in hybrids and barcodes of the *Vanda* species have been reported from Thailand (Tanee *et al.*, 2012) using RAPD analysis. The study was conducted using ten native Thai species and dendrogram was constructed from RAPD marker analysis. The results obtained from the study showed that identical species showed monophyletic group and genetic distances that were between 0.15 to 0.17. The barcodes of all wild species studied were done by two core barcodes and the tag sequences were tested for nucleotide.

Genetic variability within and among populations of an invasive, exotic orchid was studied using RAPD and ISSR markers by (Ueno *et al.*, 2015). The study reported 13 inter-simple sequence repeat primers used to assess the genetic diversity of 152 individuals of *Oeceoclades maculata* distributed in five sampled

sites from three Brazilian states. The patterns of genetic structure found in the study may be understood considering the interaction of several probable reproductive strategies with its history of colonization involving possible genetic drift, selective pressures and multiple introductions.

Genetic diversity analysis of native *Vanda* species using RAPD markers was conducted using 12 species of genus *Vanda*, collected from different locations of India (NRCO, 2011). Out of the 110 RAPD primers screened, 84 primers were identified as polymorphic and could distinguish the *Vanda* genotypes. These 84 markers were subsequently selected for genetic diversity analysis and DNA fingerprinting of *Vanda* genotypes.

Genetic variations among *Dendrobium, Vanda, and Cattleya hybrids* were also studied by (Miano *et al.,* 2015) using RAPD markers. The study was conducted with 30 orchid accessions using the RAPD primers. Out of 30 primers screened, six were selected which gave 43 clear and bright fragments, out of which 40 fragments were considered polymorphic. The UPGMA dendrogram constructed based on RAPD analysis in 30 orchid accessions were found to be grouped in seven major clusters. Cluster II was the broad one which included 7 orchids and only a single orchid formed Cluster III. The study thus proved that the RAPD analysis has a high potential in diversity analysis of orchids.

The genetic distance and relationships of 149 accessions representing 46 species in the genus *Phalaenopsis* and four species in *Paraphalaenopsis* were studied using RAPD markers (Goh *et al*., 2004). A total of 20 random primers were screened and out of these, six random primers provided 123 polymorphic bands and zero monomorphic bands. The study thus again proved the capability of RAPD to study the relationships and to distinguish taxa up to the specific level.

Genetic diversity analysis of the endangered slipper orchid *Phragmipedium longifolium* in Costa Rica using AFLP Markers (Munoz *et al.* 2010). A total of 160 samples were analyzed with amplified fragment length polymorphism technique. The genetic diversity of *P. longifolium* in Costa Rica is

high and differentiation among sampled locations was moderate in comparison with results of studies in some other terrestrial orchid species using the same technique.

Molecular characterization and phylogenetic relationships among and within species of *Phalaenopsis* was conducted based on RAPD analysis (Niknejad *et al.*, 2009) RAPD analysis for 20 species of *Phalaenopsis* was conducted to determine their genetic distances and relationships. Among 20 different primers used for RAPD analysis, 10 primers showed polymorphism, a total of 414 polymorphic fragments were generated by 10 primers and used for correlation analysis. Thus RAPD markers can be successfully applied in grouping of important orchids for the study of molecular characterization and relationships. From the study, data acquired could be used for identification and classification of other orchid genera and oriental *Phalaenopsis*.

Molecular characterization of *Dendrobium nobile L* an endangered medicinal orchid was performed using RAPD markers (Bhattacharyya, 2014). The genetic structure of *Dendrobium nobile* from Northeast India was investigated using RAPD. The PIC value of RAPD primers was 0.74 and resolving power values ranged between 6.80 and 13.23. The results of analysis of molecular variance (AMOVA) revealed that 60 individuals belonging to six natural populations in Northeast India were clustered into two major groups. The data represented in this study suggested that the RAPD method was a valuable tool for estimation of genetic diversity and genetic relatedness of the *Dendrobium nobile* germplasm. The findings in the study were useful outcomes for germplasm conservation and formulation of new breeding strategies in *Dendrobium nobile*.

Interspecific genetic analysis of orchids in Brazil were studied using molecular markers by (Fajardo, 2014) The study reported that the polymorphic information content (PIC) and optimum number of ISSR markers (ONM) for five Laeliinae orchids were evaluate to assess genetic diversity. The phylogenetic relationships between *Cattleya granulosa*, an endangered Brazilian orchid, and

four other native Brazilian species were analyzed for genetic diversity and differentiation. The 11 selected primers generated 166 unambiguous loci (PIC- 0.354; ONM- 156). Of the five studied species, *C. bicolor* exhibited the highest level of genetic diversity (HE- 0.219); while *C. labiata* exhibited the lowest level (HE- 0.132) the study thus proved that the ISSR genetic markers are effective in detecting genetic differentiation among orchid species.

Traditional morphological and cytological characterizations were used in combination with molecular results in classification and identification of *Dendrobium* (Jaime *et al.,* 2015). They reported that, in the past two decades, promising advances have been made in taxonomy, phylogeny and breeding of *Dendrobium* species due to the intensive use of molecular markers. In this review, focus was on the main molecular techniques used in 121 published studies and discuss their importance and possibilities in speeding up the breeding of new cultivars and hybrids.

2.9.4 RAPD in other crops

The phylogenetic relationships within citrus genotypes were studied using RAPD markers (Federici *et al.*, 1997). A total of 32 accessions of citrus and three microcitrus accessions were examined by random amplified polymorphic DNA (RAPD) analysis. A measure of relative heterozygosity was estimated based on the mean of the number of fragments per individual per probe-enzyme combination (PEC) divided by total number of fragments per PEC for all non-hybrids. The data from the study showed that several accessions were probably assigned to the wrong species.

The genetic diversity assessments of some important grape genotypes in India were performed using RAPD markers. From a total of 19 informative primers, most of which could clearly distinguish between the wild and cultivated genotypes. Wild species and rootstocks showed a maximum polymorphism (94

%), followed by cultivars (90 %), and while cultivars from *V. labrusca* showed almost all were monomorphic. The study report was the first attempt to determine the genetic relationships in important grape genotypes in India using molecular markers (Tamhankar *et al.*, 2001)

Wide genetic diversity of *Rosa damascena* Mill. germplasm in Iran as revealed by RAPD analysis. The genetic relationships among 41 *Rosa damascena* accessions from various cultivation areas of Iran and one accession from Bulgaria were analyzed using 31 RAPD primers. The wide genetic variation seen for *R. damascena* in Iran indicates that Iran is a center of genetic diversity for this species and that there is a promising future for the breeding (Kiani *et al.*, 2008).

The genetic diversity studies of arecanut (*Areca catechu L.)* germplasm were studied utilizing the RAPD markers, which consisted of both indigenous and exotic accessions and were assessed using 14 polymorphic RAPD primers. The average polymorphism was 6.64 markers per primer. The results obtained from the study are crucial for developing effective management strategies for genetic improvement of arecanut (Bharath *et al.*, 2015)

Molecular marker based genetic diversity analysis of cotton accessions were performed using RAPD and SSR markers the study was conducted using 23 cultivars of Gossypium hirsutum using 10 RAPD primers, which produced 34 amplicons the number of polymorphic amplicons was found to be 18 resulting into a polymorphism equivalent to 52.9 Based on the RAPD data, the genetic similarities ranged from 54 to 96 per cent, respectively (Tyagi *et al.*, 2015).

2.10 Mycorrhizae

The relationship of orchids with fungi is relatively unique in the plant kingdom. The main group of fungi inhabiting orchid roots is Basidiomycetes, though Ascomycetes have been found (Currah *et al.*, 1997). Some of the Basidiomycetes with which orchids form a relationship are pathogenic on other

crops, e.g., *Rhizoctonia solani* Kahn (Hadley, 1982). Even within *the Orchidaceae*, symbiotic fungi of one orchid species may be pathogenic on another. However, most orchids are able to control the infection and growth of endomycorrhizal fungi. Orchid mycorrhizal fungi are usually found in the intracellular in cells of the cortex and they are confined to roots (Hadley, 1982).

The mycorrhizae form dense coils of mycelium called pelotons which are thought to be adaptations to the host cell (Hadley, 1982). Within the cell, the pelotons are surrounded by a membrane and interfacial matrix material (Peterson *et al.*, 1996). The membrane lacks adenylate cyclase activity but is otherwise similar to the plasma membrane. The orientation of microtubules and cell wall microfibril is altered during infection and may be necessary to alteration in the cytoplasm and synthesis of the membrane surrounding the pelotons (Peterson *et al.*, 1996).

2.10.1 Mycorrhizal interaction with orchids

The orchid seeds are infected by the fungi at early stage of germination. The swollen and imbibed embryos are rapidly colonized by the hyphae (Smith and Read., 2008). The fungus will spread quickly from cell to cell and form coiled hyphae pelotons within the infected embryo that consists of few hundred cells. The infection is limited to the suspensor cells of the embryo and epidermal hairs and highly restricted compared to the fungal infections found in other plants. This suggests that the orchids control the infection process and the fungal symbiont are adapted to this control (Hadley, 1982). Each intercellular pelotons has a short life span and they are digested and consumed by the orchid cells while the surviving fungal hyphae colonize in adjacent cells. The orchid cells releases orchinol, a phytoalexin that causes the pelotons to collapse. This controls the degree of fungal colonization and nutrient uptake thus preventing parasitism by the fungi (Brundrett *et al.*, 2001, Smith and Read, 2008).

2.10.2 Role of Mycorrhizal Association

Role in the ecosystems

Soil hyphae have an important role in nutrient cycling by helping to prevent losses for the system, especially at the times when the roots are inactive (Lussenhop and Fogel., 1999)

Hyphae are conduits that may transport carbon from the plant roots to the soil organism involved in nutrient cycling process thus cooperating with other members of the decomposition soil food web (Smith and Read, 2008). Soil hyphae may have an important role in nutrient cycling by acquiring nutrients from saprophytic fungi (Lindahl *et al.*, 1999). Mycorrhizal fungi contribute to carbon storage in soil by altering the quality and quantity of the soil organic matter (Rygiewicz and Andersen, 1994)

2.10.3 Benefits to the plant by the mycorrhizae

Increased plant nutrient is provided by the fungal hyphae to the external roots of orchids. Increases plant nutrient supply by acquiring the nutrients form that would be not available to plants (Tarafdar and Marschner, 1994; Kahiuto and Vestberg, 1998). Significant amount of carbon transfer trough fungus mycelia connecting different plant species. Nutrient transfer from the dead and decay to living plants may occur (Eason *et al.*, 1991).

2.11 Conservation of Orchids

Orchidaceae consists of highest number of the threatened species (Swarts and Dixon 2009). Orchids species are under major threat worldwide than any other plant family due to over exploitation by the collectors and enthusiasts (IUCN, 1999). They are highly vulnerable to change in the eco system equilibrium such as availability of the nutrients, light, water. Apart from the physical factors many other threats to orchids comes as a result of various human activities. This includes clearing land for the agriculture purposes, mining, and the

urban development, weed invasion, grazing of animals and collection of the plants for the medicinal horticultural and ethno biological reasons (Lokesha and Vasudevan., 1992 Sosa and Plates., 1998). The habitat fragmentation results in the removal of the key species from the particular eco system. Susceptibility to fire threats, pollinators decline, and introduction of undomesticated animals are results in drastic losses in the diversity of orchid's population (Sosa and platas, 1998). Smaller size of the population and isolation due to habitat destruction and degradation leads in to the significant losses of the unique evolutionary linages (Coates, 2000; Hopper, 2000).

According to Swarts and Dixon (2009), effective conservation strategies must be developed to avoid further loss of essential species and ecosystem.During the formulation of policies for the conservation programmes in orchids, several aspects such existing and future threats, taxonomic distinctiveness, geographic distribution, habitat specialization, reproduction biology, evolutionary processes influencing population structure etc. should be taken into consideration. *Ex situ* conservation methods should be the key conservation aspect despite the offsite conservation methods such as seed and germplasm banks and *in vitro* propagation. *In situ* conservation and conservation *via* assisted migration are premier approaches for biodiversity conservation (Bormann *et al.*, 2007). Integrated conservation approach mainly depends on the understanding of the ecological and genetic studies, *in situ* research and *ex situ* propagation (Ramsay and Dixon, 2003).

2.11.1 Agencies for Conservation

National Botanical Research Institute (NBRI) at Lucknow (India) is one of the significant plant based national laboratory in India under the Council of Scientific & Industrial Research (CSIR), New Delhi. It was originally set-up by Government of Uttar Pradesh during 1948 as National Botanic Gardens (NBG) and later on taken over by CSIR in 1953.

The Botanic Garden at NBRI has been well known all over the world. It is the third largest and one of the oldest Botanic Gardens in India, besides Indian Botanic Garden, Howrah and Lalbagh Gardens, Bangalore. Spread over in an area of 25 hectares, it is located in the heart of Lucknow, the capital city of Uttar Pradesh along southern bank of river Gomti. It is reputed for its well identified and aesthetically displayed plant wealth to capture a living nucleus of various plant species for posterity. Botanic Garden is also a member of BGCI, U.K. and International Association of Botanic Gardens (IABG). A repository of germ plasm collection of various tropical and sub-tropical plant species, comprising 5,000 taxa, representing 212 families, the Botanic Garden has rich genetic treasure with the collection of trees, shrubs and herbs of ornamental, economic, medicinal, aromatic and rare importance, hailing from the indigenous and exotic sources.

The list of plants banned or restricted for export from India formerly included a few orchids but now include all orchids growing wild. The convention of International Trade in Endangered Species of Fauna and Flora (CITES), ratified by India, places all species of *Orchidaceae* that their trade will be only through export permits. Steps have also been taken to conserve Indian native species by establishing Orchidaria, sanctuaries and germplasm conservation centers.

Botanical survey of India (BSI) has established two Orchidaria one at Shillong and other at Yercaud to conserve rare and endangered species. The ICAR research complex at Shillong, the Indian Institute of Horticultural Research at Hessaraghatta and the Indian Botanic Gardens at Calcutta maintain collections of orchids in their Orchidaria. Some states have also established orchid sanctuaries in Sikkim at Singtom and Deorali and in Arunachal Pradesh at Tapi.

The concept of in situ conservation in the wild condition of the existing rich orchid flora at their nativity is rather lacking. There should be selection of areas rich in orchids as 'orchid preserves' at sectorial levels in the hot spot areas to prevent deforestation, habitat destruction, and indiscriminate collection by orchid lovers and exploitation by tradesman..

2.12 Economic importance

Orchid plants that exhibits an incredible range of diversity in there colour, shape, structure and fragrance of the flower (Thomas and Michael, 2007). Orchid species are endangered and some of them may have not yet been found or discovered, because of the loss of habitat resulting from fire, forest damage, illegal logging, and lack of awareness among people about the value of orchids. In addition to natural forests, the coffee based agro-forestry systems support substantial number of orchids. There is a wide range of utility of orchids like fresh bulbs of *Coelogyne asperata* are used in black board erasers in Sumatra (Wityhner, 1959); yellow pseudo stems of *Dendrobium* are used by the tribal people in the New Guinea (Bose et.al, 1999). In Philippines, Indonesia and New Guinea the *Dendrobium* stems are used for the making baskets (Yadav and Bask, 1998)

In the emergence situation, the orchids have been used as food by roasting bulbs of *Gastroidia sesamoides* (Lawler and Slaytor, 1970). Powdered roots of *Vanda tessellates* are used as antidote of the poisoning and abdominal complaints (Amin *et al*., 2004). The roots of these species have the medicated oils used for the treatment of the rheumatic swelling and nervous disorders (Hussain1992; Yoganarasimhan, 1996). Stem paste of the *Vanilla walkeriae* is given as a fodder to the cattle's (Balasubramanian *et al.,* 2000)

The orchids are valued for the cut flowers due to their wonderful name of the flowers and extended keeping quality .The orchids are mostly grown in the parks and the gardens and urban areas for beautification. (Borys *et al.,* 1999; Lopez and Runkle, 2005). Orchid flower can long last upto one –three months depending on the attachment to the plant and some of the cut flower can also last to one –four weeks.

In India, commercially growing of orchids is limited as it is in the hands of the dealers who collect orchids from the wild population (Rao 1977). Many of the Indians orchid species like *Dendrobium, Phalaenopsis, Cymbidum* and *Vanda* are

currently available in the international markets it had been reported that approx 70% of orchids plants are illegal imported by international market (Chadha, 1992).

Apart from the ornamental value, some of orchid species are used as an ingredient in traditional medicines. Some of the species of orchids such as *Arethusa bulbosa, Goodyera pubescens, Vanda hookeriana,* and *Cymbidium giganteum* are used against the treatment for toothache, joint pains, and blood clotting respectively.

Materials and Methods

3. MATERIALS AND METHODS

The present study on 'Characterisation and conservation of promising genotypes of orchids from Central Western Ghats' was carried out as a part of M.Sc.(Ag) programme of the Department of Plant Breeding and Genetics, College of Agriculture (CoA) Padannakkad, Kerala Agricultural University during the period from 2014 to 2016. Details of the experimental material and methodology used for the study are presented in this chapter.

3.1 MATERIALS

3.1.1 Area of survey

The survey and sampling of the present study was mainly conducted in the Central Western Ghats region, which is well known for the high flora and fauna diversity. The study was mainly concentrated on the above mentioned area due to the various reports and previous studies conducted in these areas Central Western Ghats (figure 1) which comprise the area extending from the south of Goa up to the Palghat gap and is approximately 360 km long and well known as the hotspot of many orchid species. Due to the administrative and logistic reasons, the survey and sampling were restricted mostly to the political boundaries of Kerala and Karnataka states as it was conducted with the support from College of Forestry, Ponnampet, University of Agricultural and Horticultural Sciences (UAHS), Shivmoga. The sampling was conducted during different time frames of 2014-2016.

3.1.2 Orchids rescued and conserved

During field survey, different species of orchids for which the existence in the natural locations was threatened, were rescued and brought carefully to the College of Agriculture, Padannakkad. Apart from these, the collection included 3 genera brought from Gangtok, Sikkim (*Vanda, Coelogyne, and Cymbidium*), one species each of *Dendrobium* and *Phalenopsis* tissue cultured plants maintained at Department of Plant Biotechnology. One species of *Dendrobiums* and *Vanda* were

collected from Padannakkad natural habitat and 9 hybrids, six from *Dendrobium*, one each from Mini *Cattleya*, *Oncidium, and Phalaenopsis* were brought from Bengaluru. These 46 accessions from different natural habitats were documented and maintained in the orchidarium attached to Department of Plant Biotechnology and were subjected to further characterization (Table1).

Table1. Orchid accessions conserved in orchidarium at College of Agriculture, Padannakkad

Sl. No.	Genus	No. of accessions conserved	Species	Accession code	Location
1	*Acampe*	1	*Acampe praemorsa*	PDK/ORP-45	Nagarhole
2	*Aerides*	1	*Aerides maculosa*	PDK/ORP-30	Mayamudi
3	*Bulbophyllum*	2	*B. elongatum*	PDK/ORP-29	Thithmathi
			B. fischeri	PDK/ORP-31	Brahmagiri
4	*Cattleya*	1	Unknown	PDK/ORP-22	Gonnikoppa
5	*Coelogyne*	2	Unknown	PDK/ORP-9	Sikkim
			C. breviscapa	PDK/ORP-28	Thithmathi
6	*Cymbidium*	6	Unknown	PDK/ORP-7	Sikkim
			Unknown	PDK/ORP-24	Sikkim
			Unknown	PDK/ORP-25	Sikkim
			Unknown	PDK/ORP-26	Sikkim
			Unknown	PDK/ORP-27	Sikkim
			Unknown	PDK/ORP-35	Pushpagiri
7	*Dendrobium*	10	Unknown	PDK/ORP-8	Padannakkad
			hybrid 1	PDK/ORP-11	Bengaluru
			hybrid 2	PDK/ORP-13	Bengaluru
			hybrid 3	PDK/ORP-15	Bengaluru
			hybrid 4	PDK/ORP-16	Bengaluru
			hybrid 5	PDK/ORP-17	Bengaluru
			hybrid 6	PDK/ORP-18	Bengaluru
			Unknown	PDK/ORP-20	Brahmagiri
			D. aqueum	PDK/ORP-34	Brahmagiri
			Unknown (T.C)	PDK/ORP-42	Padannakkad
8	*Flickingeria*	1	*Flickingeria nodosa*	PDK/ORP-39	Pushpagiri
9	Mini *Cattleya*	1	Mini *Cattleya*	PDK/ORP-14	Bengaluru
10	*Oncidium*	1	*Oncidium tolumina*	PDK/ORP-12	Bengaluru
11	*Phalaenopsis*	2	Hybrid	PDK/ORP-10	Bengaluru
			unknown (T.C)	PDK/ORP-38	Padannakkad

12	*Pholidota*	1	*Pholidota imbricata*	PDK/ORP-32	Brahmagiri
13	*Rhynchostylis*	2	*R. retusa*	PDK/ORP-33	Brahmagiri
			R. retusa	PDK/ORP-37	Ponnampet
14	*Vanda*	15	*Vanda testacea*	PDK/ORP-1	Nagarhole
			Unknown	PDK/ORP-2	Nagarhole
			Unknown	PDK/ORP-3	Nagarhole
			Unknown	PDK/ORP-4	Thithmathi
			Unknown	PDK/ORP-5	Thithmathi
			Unknown	PDK/ORP-6	Sikkim
			Unknown	PDK/ORP-19	Sullia
			Unknown	PDK/ORP-21	Nagarhole
			Unknown	PDK/ORP-23	Thithmathi
			Unknown	PDK/ORP-36	Brahmagiri
			Unknown	PDK/ORP-40	Pushpagiri
			Unknown	PDK/ORP-41	Pushpagiri
			Unknown	PDK/ORP-43	Nagarhole
			Unknown	PDK/ORP-44	Nagarhole
			Unknown	PDK/ORP-46	Padannakkad
		Total = 46			

(T.C=Tissue cultured plant)

3.1.3 Laboratory chemicals and glassware used for molecular characterization

The chemicals used in the study were from Merck India Ltd. and SRL laboratories. The *Taq* DNA polymerase along with buffer, dNTPs and molecular weight markers (lambda DNA *Eco RI/ Hind III* double digest, 100 bp ladder, and 1 kb ladder) were from Merck-Genie, Bangalore. The random decamer primers were selected based on earlier reports and the oligos were synthesized by Integrated DNA Technologies, New Delhi.

3.1.4 Equipments and machinery used for molecular characterization

The equipments available in the Department of Plant Biotechnology, College of Agriculture Padannakkad were used for the study. DNA quantification was done using biophotometer (Eppendorf, Germany) and Polymerase Chain

Reaction (PCR) was done in Master cycler Eppendorf, (Germany). Bio-Rad imaging system was used for imaging and documenting the agarose gel.

3.2 METHODS

3.2.1 Survey

A survey was conducted in Central Western Ghats, to assess and understand the current status of orchid diversity of the region. The selected region included six major habitats, *viz.*

1. Aralam Wildlife Sanctuary, Kannur, Kerala
2. Ranipuram Wildlife Sanctuary,Kasaragod, Kerala
3. Pushpagiri Wildlife Sanctuary, Kodagu, Karnataka
4. Brahmagiri Wildlife Sanctuary, Kodagu, Karnataka
5. Talakavery Wildlife Sanctuary, Kodagu, Karnataka
6. Rajeev Gandhi / Nagarhole National park, Nagarhole, Karnataka

Within each habitat 4 transect of one kilometre length was marked and in each transect, at every 100 m, 40×40 m quadrates were made. Within the quadrates enumeration of epiphytic and ground orchids was done. The data was analyzed and represented on an average basis. The data was later subjected to Shannon's and Simpson's diversity index (Ambinakudige *et al.*, 2008)

3.2.2 Micro climatic observations

Micro climatic observations recorded in different natural habitat of the Central Western Ghats include habitat, location, latitude, longitude, elevation, and light intensity temperature inside the habitat, temperature in canopy, relative humidity, and wind speed. The weather data obtained from Department of Meteorology Regional Agricultural Research Station (RARS), Pilicode were also included in the study, as the rescued accessions were maintained in a different condition at CoA, Padannakkad, compared to the natural habitat.

3.2.3 Identification of orchid species and establishment in orchidarium

The collected orchid species were identified by using standard literature including local/regional floras and revision/monograph (Cooke, 1967; Abraham and Vatsala, 1981; Rao, 1986; Nageswara Rao, 1986; Bose et al., 1999; Manilal and Sathish Kumar, 2004). Additionally, the identification was also accomplished with the help of orchid experts working at College of Forestry, Ponnampet.

Epiphytic orchids were planted on to the barks of mango tree logs wrapped with gunny bags, locally available mosses, coir pith etc. for proper adhering of roots and to prevent moisture. Some of the terrestrial orchids were planted in 1:1:1 mix of soil, vermiculate and cattle manure; whereas some epiphytes were planted in mixture 1:1:1:1 of bark, brick pieces, wood, and charcoal.

The accessions were denoted by alphabetical letter and numbers (PDK/ORP-1), the first three letters representing Padannakkad (PDK) and the next three letters representing orchid plant (ORP). The number represents the serial number of different accessions present in the orchidarium, as and when they are collected (Table 1).

3.2.4 Morphological characterization

The vegetative and reproductive characters of the 46 accessions present in CoA, Padannakkad were recorded during 2015-2016 (November to June) with an interval of 30 days. Observations were recorded based on Descriptor available from National Research Centre on Orchids, Sikkim.

The details of the observations recorded are shown below.

3.2.4.1 Vegetative characters

Plant height (cm)

Total height of plant was measured from the base of plant to tip of apex leaf in centimeter (cm).

Total number of leaves

Number of leaves present on the plant at the time of recording the observation.

Internodal length (cm)

Average length of 3 internodes just above the base of plant measured in centimeter (cm).

Leaf length (cm)

Average length of 3 leaves measured from petiole to the tip of leaf in centimeter (cm).

Leaf width (cm)

Average width of 3 leaves measured at the centre of leaf in centimeter (cm).

Total no of sprouts

Total number of new sprouts present at the time of recording of observation.

Nature of pseudo bulb

Nature of pseudo bulb was described according to NRCO descriptor.

Shape of leaf

Shape of leaf was described according to NRCO descriptor.

Leaf apex

Leaf apex was described according to NRCO descriptor.

Based on the observations as per the respective descriptor for each genus. Plants were classified very small, small, medium, large, very large, long, broad, few, and many

Absolute growth parameters

Absolute growth parameters were recorded from November 2015 to June 2016.The absolute growth rate was calculated by using formulae

$$\text{Absolute growth (\%)} = \frac{B-A}{A}$$

3.2.4.2 Observation on reproductive characters

The observations on reproductive characters of 6 accessions which flowered during the period were recorded at flowering phases, viz. *Dendrobium* Hybrid 1, Hybrid 2, Hybrid 3, Hybrid 4, *Dendrobium, and Acampe praemorsa* (PDK/ORP-11, PDK/ORP-13, PDK/ORP-15, PDK/ORP-16, PDK/ORP-20, and PDK/ORP-45 respectively).

Sample collection

Inflorescences were collected from the orchidarium at CoA Padannakkad and morphological observations were noted immediately. The length and width of the petals were measured using vernier caliper. From the flower the pollinia was collected carefully and photographed using Leica stereo zoom microscope as per standard protocol (Chaudhury *et al.*, 2012).

Number of petals

Total number of petals present in a flower was recorded.

Number of dorsal sepals

Total number of dorsal sepals present in a flower was recorded.

Number of ventral sepals

Total number of ventral sepals present in a flower was recorded.

Length and width of petal (cm)

Maximum length and width of petal were measured in centimeter (cm). Observations are average value of 3 flowers.

Length and width of dorsal sepal (cm)

Maximum length and width of dorsal sepal were measured in centimeter (cm). Observations are average value of 3 flowers.

Length and width of ventral sepal (cm)

Maximum length and width of ventral sepal were measured in centimeter (cm). Observations are average value of 3 flowers.

Lip length and width (cm)

Maximum length and width of lip were measured in centimeter (cm). Observations are average value of 3 flowers.

Petal shape

Petal shape was described according to NRCO descriptor.

Petal curvature

Petal curvature was described according to NRCO descriptor.

Dorsal sepal shape

Dorsal sepal shape was described according to NRCO descriptor.

Lateral sepal shape

Lateral sepal shape was described according to NRCO descriptor.

Lip shape

Lip shape was described according to NRCO descriptor.

Lip margin

Lip margin was described according to NRCO descriptor.

Apex of petal

Apex of petal was described according to NRCO descriptor.

Apex of dorsal sepal

Apex of dorsal sepal was described according to NRCO descriptor.

Apex of lateral sepal

Apex of lateral sepal was described according to NRCO descriptor.

Apex of lip

Apex of lip was described according to NRCO descriptor.

Lip surface texture

Lip surface texture was described according to NRCO descriptor.

Flower duration (Days)

The period in days between the opening of the first flower and shedding of the last flower in the inflorescence was recorded.

3.2.4.3 Palynological observation

Number of pollinia

Total number of pollinia present in a flower was recorded.

Pollinia shape

Pollinia shape was determined based on Stenzel (2000), Chaudhary *et al.* (2012).

Pollinia colour

Pollinia colour was described according to the Royal Horticultural Society colour chart.

Pollen grain shape

Pollen grain shape was determined based on Stenzel (2000), Chaudhary *et al.* (2012).

Pollen viability

The pollinia removed from flowers were placed on a microscope glass slide. A few drops of safranin 0.01% were added on the material which was then squeezed using a glass rod. After putting the cover slip, the prepared samples were examined under Leica M80 stereo zoom microscope, at 40× magnification. The photographs of pollinia were captured, using software Leica Application Suite (LAS) V4.4. Number of viable pollen grains was determined by taking average of five fields under the microscope.

3.2.5 Molecular characterization

3.2.5.1 DNA isolation

The leaves were collected for isolation of genomic DNA from the samples. The young tender leaf was used for DNA extraction. Leaf samples from the respective genotypes were brought to the laboratory using zip-lock plastic bags and wiped with 70% alcohol to remove the dirt adhering to the leaf tissue.

Genomic DNA was extracted from the leaf samples using the in-house protocol (conventional) method available at Department of Plant Biotechnology, CoA Padannakkad, modified from Rogers and Bendrich (1994) and the kit method (Origin Diagnostics, Kerala).

In the conventional method of DNA extraction, the leaf samples were used to extract DNA using Cetyl trimethyl ammonium bromide (CTAB) method. The various steps involved in the DNA isolation are as follows.

CTAB extraction buffer:

100 mM	Tris (pH 8)
20 mM	EDTA
1.4 mM	Nacl
02%	CTAB

1× TAE Buffer:

40 mM	Tris
20 mM	Acetic acid
1 mM	EDTA

Steps involved:

i) Take 1gm leaf tissue in cold mortar and pestle and add 25µl β-mercaptoethanol, with a pinch of PVP and grind with liquid nitrogen.

ii) Immediately add 5ml of pre heated (60^0C-65^0C) extraction buffer and transfer to oak ridge centrifuge tube. Mix thoroughly by inverting the tube for several times and incubate the tube in hot water for 65^0C for 30 mins. Occasionally mix by inverting slowly.

iii) Add equal volume of chloroform: isoamyl alcohol to the tube. Mix by inverting for several minutes to emulsify and then centrifuge for about 12,000 rpm for 15 mins.

iv) Pipette out the upper aqueous phase, add 0.5 volumes of chilled 5M Nacl and 1.5 volumes of 95% alcohol.

v) Invert the tube slowly for several times so that the DNA precipitates. Keep it in freezer for 10 min for complete precipitation. Centrifuge at 10,000 rpm for 5 mins.

vi) Decant the solution leaving the pellet in tube and wash with the 70% alcohol.

vii) Air dry the DNA pellet in room temperature till alcohol evaporates.

viii) Dissolve the pellet in 100 µl of sterile water.

In the kit method of DNA extraction, the protocol instructed by the manufacturer was followed as described below:

i) Grind 300mg fresh leaf tissue using liquid nitrogen.

ii) Add 2.1 ml 65^0C preheated GP1 (β-mercaptoethanol must be added to buffer GP1 before use. The final concentration is 0.1%) to the powdered plant tissue. Vortex for 10-20sec to mix, make sure to disperse all clumps and then incubate for 20 min at 65^0C, mix by inverting the tube for several times.

iii) Add 2.1 ml chloroform, mix by inverting the tube for several times, centrifuge for 5 min at 12,000 rpm.

iv) Pipet the supernatant to a new tube, add 2.1 ml buffer GP2, mix by inverting the tube for several times.

v) Pipet all the mixture from step 4, including any precipitate that may have formed, into the spin column CB3 (place the spin column CB3 in the collection tube). Close the CB3 lid and centrifuge for 30 Secs at 12,000 rpm. Discard the filtrate and set the spin column CB3 into the collection tube. Since the capacity of CB3 is 700 µl, centrifuge step should be repeated several times for processing all the mixture from step 4.

vi) Carefully open the column and add 500 µl buffer GD, close the lid and centrifuge at 12,000 rpm for 30 Secs then discard the filtrate and place the spin column into the collection tube.

vii) Add 700 µl PW to the spin column CB3 to wash the membrane, and centrifuge for 30 Secs at 12,000 rpm, discard the flow-through, and replace the spin column CB3 in the collection tube.

viii) Add 500 µl PW to the spin column CB3 to wash the membrane and centrifuge for 30 sec at 12,000 rpm, discard the flow-through.

ix) Replace the spin column CB3 in the collection tube, centrifuge for 2 mins at 12,000 rpm to remove residual wash buffer PW. Discard the collection tube and transfer the spin column CB3 to a clean 1.5 ml or 2 ml micro centrifuge tube. Open the lid of the CB3 and incubate the assembly at room temperature (15-20^{0}C) or 50^{0}C for several minutes to dry membrane completely.

x) Pipet 50-200 µl buffer TE directly onto the CB3 membrane, incubate for 2-5 mins at room temperature, and then centrifuge for 2 mins at 12,000 rpm to elute.

3.2.5.2 Quantity and quality analysis of DNA

The quality of the DNA samples were determined by observing the ratio of absorbance of UV rays at 260nm and 280nm by using Eppendorf Biophotometer. The DNA was also further checked by subjecting it to 0.8% agarose gel electrophoresis (Sambrook, 1989).

The steps involved in Agarose gel electrophoresis for the quality analysis of DNA is mentioned below:

i) *Set-up the casting trays and apparatus:* Wash the gel casting tray and comb with water to remove dirt. After drying wipe off with 70% ethanol. Close the open ends of the trays with cellophane tape while the gel is being cast, then remove prior to electrophoresis. Place the apparatus on a level surface and check with the spirit level and adjust the level. Place the gel tank on a level surface and check with the spirit level and adjust the level.

ii) *Dissolve the agarose in Tris-acetate EDTA (TAE) buffer:* To 1 gm of agarose, add 100 ml of 1X TAE. Heat until the agarose is dissolved in a microwave oven. Cool the gel to 50°C and add EtBr (0.5 pg/ml) and mix by swirling before pouring into the gel apparatus.

iii) *Pouring the gel:* The casting trays should be fitted with properly cleaned combs. The gel should be poured onto the casting trays without making any air bubbles. Allow the gel to solidify at room temperature. After solidification, the combs are removed gently by pouring little amount of buffer.

iv) *Loading the samples:* Immense the gel slowly into the gel tank. Connect the electrodes to the power pack. Load the samples (1 µl of 6× gel loading dye + 5 µl of DNA) carefully into the wells without poking the gel. An aliquot of standard molecular weight marker should be also added to assess the size of DNA fragment. Electrophoresis at 70 volts until dye has migrated two third the length of the gel. The gel is visualized in a gel documentation unit.

The steps involved in the quality analysis of isolated DNA, using a biophotometer is mentioned below:

i) Set the 'Blank' using sterile distilled water in the cuvette.

ii) Dilute 5 µl of the stock DNA 10 times by adding 45 µl of sterile distilled water.

iii) Insert the cuvette with sample (diluted DNA) and press 'Sample'.

iv) Record the values in the digital display (given in µg/mL, equivalent to ng/µl).

3.2.5.3 Primer screening for RAPD

For RAPD analysis, 30 random decamer primers were initially screened using genomic DNA from two different orchid genera *viz., Phalenopsis* (PDK/ORP-10) and *Cymbidium* (PDK/ORP-24). Out of the thirty primers tested for their efficiency in detecting polymorphism between the accessions, 10 were

selected for characterizing the 14 genotypes based on the intensity and clarity of the amplicons as well as extent of polymorphism brought out between the two genera (Table 2).

Table 2. Screening of RAPD primers for amplifying orchid genomic DNA

Sl. No.	Primer	Nucleotide sequence	Primer selection
1	OPA05	5'-AGGGGTCTTG- 3'	selected
2	OPA10	5'-GTGATCGCAG- 3'	
3	OPA11	5'-CAATCGCCGT- 3'	selected
4	OPA14	5'-TCTGTGCTGG- 3'	
5	OPA16	5'-AGCCAGCGAA- 3'	selected
6	OPA17	5'-GACCGCTTGT- 3'	
7	OPA18	5'-AGGTGACCGT- 3'	selected
8	OPA20	5'-GTTGCGATCC- 3'	selected
9	OPAU02	5'-CCAACCCGCA- 3'	
10	OPAU03	5'-ACGAAACGGG- 3'	
11	OPAW09	5'-ACTGGGTCGG- 3'	
12	OPAW11	5'-CTGCCACGAG- 3'	
13	OPAW12	5'-GAGCAAGGCA- 3'	selected
14	OPAW13	5'-CTACGATGCC- 3'	
15	OPAW14	5'-GGTTCTGCTC- 3'	
16	OPAW18	5'-GGCGCAACTG- 3'	
17	OPAW05	5'-CTGCTTCGAG- 3'	selected
18	OPB01	5'-GTTTCGCTCC- 3'	
19	OPB05	5'-TGCGCCCTTC- 3'	
20	OPB12	5'-CCTTGACGCA- 3'	
21	OPBA3	5'-GTGCGAGAAC- 3'	selected
22	OPC17	5'-TTCCCCCCAG- 3'	
23	OPD07	5'-TTGGCACGGG- 3'	
24	OPE12	5'-TTATCGCCCC- 3'	selected
25	OPH2O	5'-GGGAGACATC- 3'	
26	OPM12	5'-GGGACGTTGG- 3'	
27	OPM15	5'-GACCTACCAC- 3'	selected
28	OPM20	5'-AGGTCTTGGG- 3'	
29	OPX15	5'-CAGACAAGCC- 3'	
30	OPX17	5'-GACACGGACC- 3'	

3.2.5.4 PCR conditions for RAPD reaction

Amplification was performed in a total volume of 25 µl including 2.5 µl of Taq DNA polymerase buffer, 30ng of DNA, 150mM dNTPs, 1.5pm primer and, 1U Taq DNA polymerase. PCR amplification was performed in an Eppendorf Master Cycler with the PCR reaction profile optimized as given below,

- Initial denaturation – 92^0C for 5 min
- Final denaturation – 92^0C for 1 min
- Primer annealing – 37^0C for 1 min
- Primer extension – 72^0C for 2 min

} 36 cycles

- Final extension – 72^0C for 10 min

– 4^0C - hold the sample

Amplification products were separated by electrophoresis on 1.5% agarose gel and in 1× TAE buffer (pH 8.0) stained by ethidium bromide Sambrook *et al.* (1989).

3.2.5.5 Gel documentation and data analysis

The gel documentation was carried out using BIO-RAD imaging system. The gel picture was examined for intactness, and clarity of band. Amplified products were scored as present (1) or absent (0) to form a binary matrix. Data were analyzed based on the Jaccard's Sorensen-Dice and Simple matching similarity coefficients for binary data via SIMQUAL of the Numerical taxonomy and Multivariant Analysis System program Package for PC (NTSYS-pc ver. 2.021i Package) (Rohlf, 1999). UPGMA dendrograms were constructed based on the analysis of the data (Sokal and Sneath, 1963).

3.2.6 Mycorrhizal association studies

3.2.6.1 Sample collection

From the 46 accession of orchids present in the orchidarium at CoA Padannakkad, a total of 14 accessions belong to different species were selected for

the mycorrhizal association studies. Root samples of 14 genotypes of orchids were collected.

Equipments and reagents:

1. Root samples
2. Scissors and needles
3. Petri dish
4. Glass slides and cover slips
5. Compound microscope
6. FAA : formalin: acetic acid: alcohol (1:9:9 v/v/v))
7. 10% KOH
8. 1% HCl
9. 0.05% tryphan blue in lacto phenol (lacto phenol is prepared by combining 250 ml lactic acid, 300 ml phenol and 250 ml glycerine and 300 ml water).

Procedure:

1. Root samples were collected from orchidarium and preserved in FAA.
2. Roots were washed in running tap water thoroughly to remove any dirt particles.
3. Roots were chopped equally to 1 cm in length.
4. 10% KOH solution was added to cover the roots. Root sample were autoclaved at 121^0C at 15 Psi for 15 min (duration depends on the type of the roots being evaluated).
5. KOH was poured off and roots were rinsed with tap water at least 3 times until no brown colour appeared in the rinsed water.
6. The roots were covered with 1%HCl and soaked for 3-5 mins and solution was poured off (do not rinse after this step, the specimen must be acidified for the proper staining).
7. Roots were incubated over night with the staining solution (0.05% tryphan blue in lacto phenol).

8. Roots were placed for destaining in lacto glycerol.
9. Roots were observed for the infection using compound microscope at a magnification of 400× and photographed.

Results

4. RESULTS

An investigation on 'Characterisation and conservation of promising genotypes of orchids from Central Western Ghats' was carried out at the Department of Plant Breeding and Genetics, College of Agriculture Padannakkad, Kerala Agricultural University during the period from 2014 to 2016. The results obtained from different experiments are presented in this chapter.

4.1 SURVEY

The survey was conducted in Central Western Ghats covering two protected areas in Kerala namely Aralam Wildlife Sanctuary, Ranipuram Wildlife Sanctuary and also adjoining areas of the Kodagu district of Karnataka located in Central Western Ghats which included the Pushpagiri Wildlife Sanctuary, Brahmagiri Wildlife Sanctuary, Talakavery Wildlife Sanctuary and Rajeev Gandhi / Nagarhole National Park. The survey was covering four transects of one Kilometre length each. In each transect at every 100 m, a 40×40m quadrates were made and within the quadrates enumeration of epiphytic and ground orchid was done. The survey conducted has come across a total of approximately 9463 accessions of orchids belonging to 30 genera and 70 species. The results obtained during the survey conducted during 2014-2016 are summarized below (Figure 1) (Plate 1 a, b).

4.1.1 Aralam Wildlife Sanctuary

The survey conducted at the Aralam Wildlife Sanctuary mainly focused on the evergreen/semi evergreen/shola type vegetation. The survey covered a total of 1194 accessions, which represents about 24 species coming under 15 genera. Majority of the accessions surveyed belong to the genus *Bulbophyllum* (202 accessions), of which highest number of accessions were observed in the species *Bulbophyllum elangata* (87 accessions).

The details of the various species along with with Shannon and Simpson diversity index are given in Table 3.

Table 3. Orchid diversity at Aralam Wildlife Sanctuary

Sl. No.	Species	No. of Accessions	Shannon Index	Simpson Index
1	*Acampe praemorsa*	132	0.243466	17292
2	*Aerides maculosa*	31	0.094793	930
3	*Bulbophyllum elangata*	87	0.190843	7482
4	*Bulbophyllum fimbriatum*	61	0.151948	3660
5	*Bulbophyllum fischerii*	54	0.140024	2862
6	*Cleisostoma tenuifolium*	32	0.097000	992
7	*Coelogyne mossaie*	17	0.060537	272
8	*Coelogyne nervosa*	39	0.111758	1482
9	*Cottonia pedunclaris*	15	0.054988	210
10	*Cymbidium bicolor*	48	0.129201	2256
11	*Dendrobium herbaceum*	36	0.105574	1260
12	*Dendrobium macrostachyum*	121	0.231995	14520
13	*Dendrobuim heyneanum*	12	0.046233	132
14	*Eulophia epidendrea*	78	0.178234	6006
15	*Habenaria diphylla*	98	0.205201	9506
16	*Habenaria longicorniculata*	21	0.071065	420
17	*Habenaria plantaginea*	24	0.078533	552
18	*Malaxis acuminata*	34	0.101337	1122
19	*Malaxis rheedi*	54	0.140024	2862
20	*Oberonia denticulate*	24	0.078533	552
21	*Rhynchostylis retusa*	69	0.164754	4692
22	*Trias stocksii*	39	0.111758	1482
23	*Vanda spathulata*	19	0.065889	342
24	*Vanda tessellata*	49	0.131046	2352
	TOTAL	1194	2.984732	83238

4.1.2 Ranipuram Wildlife Sanctuary

The preliminary survey conducted at the Ranipuram Wildlife Sanctuary mainly focused on the tropical evergreen/shola type vegetation. The area has a total of 670 accessions, which represents 22 species coming under 15 genera. Majority of the accessions surveyed belong to the genus *Acampe* (461 accessions),

of which highest number of accessions were observed in the species *Acampe praemorsa* (461 accessions) (Plate 2).

The details of the various species along with Shannon and Simpson diversity index are given in Table 4.

Table 4. Orchid diversity at Ranipuram Wildlife Sanctuary

Sl. No.	Species	No. of Accessions	Shannon Index	Simpson Index
1	*Acampe praemorsa*	10	0.06276	90
2	*Aerides maculosa*	32	0.14527	992
3	*Bulbophyllum elangata*	18	0.09717	306
4	*Bulbophyllum fimbriatum*	39	0.16553	1482
5	*Bulbophyllum mysorense*	35	0.15421	1190
6	*Coelogyne breviscapa*	24	0.11926	552
7	*Coelogyne narvosa*	14	0.08083	182
8	*Cattleya*	69	0.23410	4692
9	*Dendrobium ovatum*	31	0.14220	930
10	*Dendrobium aqueum*	45	0.18138	1980
11	*Dendrobium barbatulum*	64	0.22432	4032
12	*Flickingeria nodosa*	39	0.16553	1482
13	*Liparis viridiflora*	20	0.10482	380
14	*Luisia zeylanica*	17	0.09322	272
15	*Malaxis rheedi*	15	0.08506	210
16	*Oberonia denticulate*	38	0.16276	1406
17	*Oberonia mucronata*	31	0.14220	930
18	*Pholidoto pallida*	45	0.18138	1980
19	*Rhyncostylis retusa*	21	0.10853	420
20	*Trias stocksii*	25	0.12270	600
21	*Vanda tessellata*	17	0.09322	272
22	*Vanda testacea*	21	0.10853	420
	TOTAL	670	2.97498	24800

4.1.3 Pushpagiri Wildlife Sanctuary

The survey conducted at the Pushpagiri Wildlife Sanctuary mainly focused on the ever green/shola type vegetation. The selected area has a total of 2203 accessions, which represent 34 species coming under 18 genera. Majority of the

accession belong to the genus *Dendrobium* (422 nos), of which highest number were observed in the species *Dendrobium crepidatum* (72 nos).

The details of the various species along with Shannon and Simpson diversity index are given in Table 5.

Table 5: Orchid diversity at Pushpagiri Wildlife Sanctuary

Sl. No.	Species	No. of Accessions	Shannon Index	Simpson Index
1	*Aerides maculosa*	179	0.20396	31862
2	*Bulbophyllum elangata*	67	0.106229	4422
3	*Ceologyne braviscapa*	21	0.044355	420
4	*Coelogyne narvosa*	88	0.128634	7656
5	*Cottonia pedunclaris*	77	0.117222	5852
6	*Cymbidium*	53	0.089671	2756
7	*Dendrobium ovatum*	36	0.067229	1260
8	*Dendrobium aqueum*	55	0.09213	2970
9	*Dendrobium barbatulum*	20	0.042686	380
10	*Dendrobium crepidatum*	72	0.111805	5112
11	*Dendrobium herbaceum*	71	0.110702	4970
12	*Dendrobium macrostachyum*	28	0.055484	756
13	*Dendrobium nanum*	71	0.110702	4970
14	*Dendrobium nutantiflorum*	13	0.030288	156
15	*Dendrobium peguanum*	56	0.093347	3080
16	*Diplocentrum congestum*	108	0.147829	11556
17	*Eria mysorensis*	42	0.075495	1722
18	*Eria reticosa*	42	0.075495	1722
19	*Flickingeria nodosa*	299	0.271059	89102
20	*Geodorum terrirsties*	94	0.13459	8742
21	*Habenaria longicorniculata*	21	0.044355	420
22	*Habenaria marginata*	20	0.042686	380
23	*Habenaria rariflora*	65	0.103952	4160
24	*Habenaria roxburghii*	12	0.028394	132
25	*Luisia abrahami*	49	0.084649	2352
26	*Malaxis acuminata*	35	0.065809	1190
27	*Malaxis rheedi*	64	0.102804	4032
28	*Oberonia denticulate*	78	0.118288	6006
29	*Oberonia mucronata*	53	0.089671	2756
30	*Satyrium nepalense*	53	0.089671	2756
31	*Smithonia straminea*	39	0.071415	1482
32	*Trias stocksii*	64	0.102804	4032
33	*Vanda tessellata*	26	0.052395	650
34	*Vanda testacea*	132	0.168656	17292
	TOTAL	2203	3.274464	237106

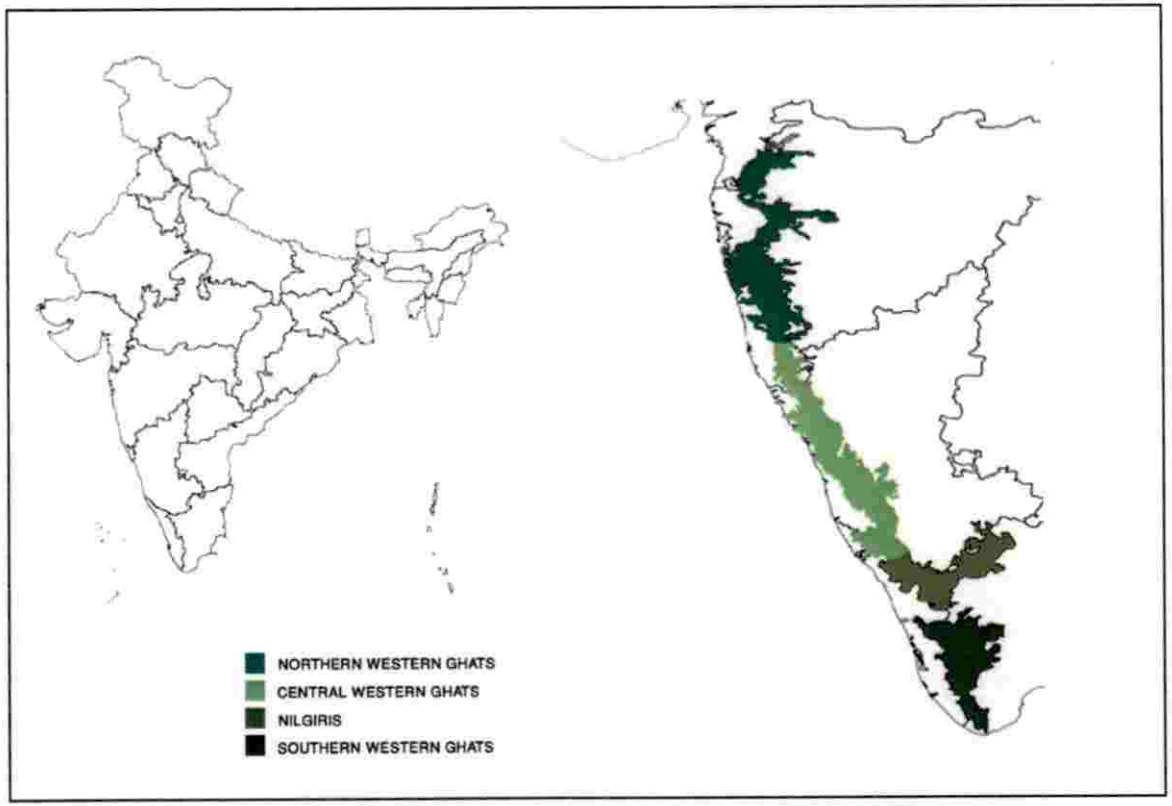


Figure 1. Map indicating the location of Central Western Ghats with parts of Western Ghats.

4.1.4 Brahmagiri Wildlife Sanctuary

The survey conducted at the Brahmagiri Wildlife Sanctuary mainly focused on the evergreen/semi evergreen/shola type vegetation. The area comprised a total of 2337 accessions which represent 39 species coming under 23 genera. Majority of the accessions surveyed belong to the genus *Oberonia* (412 accessions), of which highest number of accessions were observed in the species *Oberonia denticulate* (210 accessions) (Plate 2; Plate 3)

The details of the various species along with Shannon and Simpson diversity index are given in Table 6.

Table 6. Orchid diversity at Brahmagiri Wildlife Sanctuary

Sl. No.	Species	No. of Accessions	Shannon Index	Simpson Index
1	*Aerides maculosa*	62	0.096289	3782
2	*Bulbophyllum fischeri*	74	0.109324	5402
3	*Ceologyne braviscapa*	77	0.112446	5852
4	*Cleisostoma tenuifolium*	9	0.02141	72
5	*Coelogyne narvosa*	83	0.118543	6806
6	*Cymbidium bicolor*	107	0.141192	11342
7	*Dendrobium ovatum*	49	0.081034	2352
8	*Dendrobium aqueum*	90	0.125423	8010
9	*Dendrobium herbaceum*	44	0.074791	1892
10	*Diplocentrum congestum*	34	0.061544	1122
11	*Eria microchilos*	58	0.091732	3306
12	*Eria mysorensis*	6	0.015314	30
13	*Eria reticosa*	8	0.019434	56
14	*Eulophia pacra*	41	0.070931	1640
15	*Flickingeria nodosa*	178	0.196115	31506
16	*Habenaria rariflora*	64	0.098526	4032
17	*Liparis viridiflora*	67	0.101831	4422
18	*Luisia abrahami*	39	0.068305	1482
19	*Luisia zeylanica*	25	0.048542	600
20	*Malaxis acuminata*	11	0.025223	110
21	*Malaxis rheedi*	188	0.202736	35156
22	*Nervilia aragoana*	11	0.025223	110
23	*Nervilia cruciformis*	26	0.050048	650

24	*Oberonia brunoniana*	53	0.085869	2756
25	*Oberonia denticulate*	210	0.216516	43890
26	*Oberonia mucronata*	91	0.126386	8190
27	*Oberonia ranganniana*	17	0.035814	272
28	*Oberonia santapaiui*	41	0.070931	1640
29	*Papilionathe cylindrica*	14	0.030657	182
30	*Papilionathe subulatal*	14	0.030657	182
31	*Pecteilis gigantea*	30	0.05591	870
32	*Peristylus aristatus*	26	0.050048	650
33	*Pholidota imbricata*	69	0.104002	4692
34	*Rhyncostylis retusa*	47	0.078564	2162
35	*Satyrium nepalense*	45	0.076058	1980
36	*Vanda tessellate*	51	0.083468	2550
37	*Vanda testacea*	67	0.101831	4422
38	*Dendrobium nanum*	130	0.160711	16770
39	*Vanda thwaitesii*	81	0.116532	6480
	TOTAL	2337	3.37991	227420

4.1.5 Talakavery Wildlife Sanctuary

The survey conducted at the Talakavery Wildlife Sanctuary mainly focused on the tropical evergreen/shola type vegetation. The area comprises a total of 1426 accessions, which represents 27 species coming under 13 genera. Majority of the accessions surveyed belong to the genus *Habenaria* (317 accessions), of which highest number of accessions were observed in the species *Habenaria ovalifolium* (201 accessions).

The details of various species with Shannon and Simpson diversity index are given in Table 7.

Table 7. Orchid diversity at Talakavery Wildlife Sanctuary

Sl. No.	Species	No. of Accessions	Shannon Index	Simpson Index
1	*Aerides maculosa*	79	0.160281	6162
2	*Bulbophyllum elangata*	67	0.143676	4422
3	*Coelogyne breviscapa*	57	0.128693	3192
4	*Coelogyne narvosa*	88	0.171883	7656
5	*Cymbidium aloifolium*	12	0.040205	132

6	*Cymbidium bicolor*	41	0.102042	1640
7	*Dendrobium ovatum*	46	0.110774	2070
8	*Dendrobium aqueum*	87	0.170627	7482
9	*Dendrobium herbaceum*	58	0.130243	3306
10	*Dendrobium macrostachyum*	14	0.045393	182
11	*Eria microchilos*	16	0.050379	240
12	*Eria reticosa*	90	0.174371	8010
13	*Habenaria longicorniculata*	40	0.100245	1560
14	*Habenaria marginata*	43	0.105583	1806
15	*Habenaria ovalifolium*	201	0.276174	40200
16	*Habenaria rariflora*	14	0.045393	182
17	*Habenaria roxburghii*	19	0.057535	342
18	*Liparis viridiflora*	85	0.168091	7140
19	*Luisia abrahami*	64	0.139299	4032
20	*Luisia macrantha*	9	0.031970	72
21	*Oberonia brunoniana*	47	0.112473	2162
22	*Oberonia denticulate*	124	0.212378	15252
23	*Oberonia ranganniana*	10	0.034783	90
24	*Oberonia santapaiui*	17	0.052805	272
25	*Papilionathe cylindrica*	41	0.102042	1640
26	*Pecteilis gigantea*	42	0.103821	1722
27	*Polystachea concreta*	15	0.047909	210
	TOTAL	1426	3.019068	121176

4.1.6 Rajeev Gandhi National Park

The survey conducted at the Rajeev Gandhi / Nagarhole National Park part of kodagu district mainly focused on the tropical dry deciduous / tropical moist deciduous type vegetation. The area contains a total of 1633 accessions, which represents about 25 species coming under 16 genera. Majority of the accessions which were surveyed belong to the genus *Vanda* (363 accessions), of which highest number of accessions were observed in the species *Vanda testacea* (312 accessions) (Plate 2; Plate 3)

The details of the various species along with Shannon and Simpson diversity index are given in Table 8.

Table 8. Orchid diversity at Rajeev Gandhi National Park

Sl. No.	Species	No. of Accessions	Shannon Index	Simpson Index
1	*Acampe praemorsa*	20	0.053918	380
2	*Bulbophyllum fimbriatum*	64	0.126953	4032
3	*Coelogyne breviscapa*	27	0.067828	702
4	*Coelogyne narvosa*	12	0.036105	132
5	*Cottonia pedunclaris*	68	0.132363	4556
6	*Cymbidium aloifolium*	92	0.16205	8372
7	*Cymbidium bicolor*	218	0.268819	47306
8	*Dendrobium barbatulum*	42	0.094146	1722
9	*Dendrobium ovatum*	68	0.132363	4556
10	*Diplocentrum congestum*	31	0.075254	930
11	*Eria microchilos*	21	0.055987	420
12	*Eulophia pacra*	33	0.078846	1056
13	*Geodorum terrirsties*	76	0.142759	5700
14	*Habenaria heyneana*	134	0.205171	17822
15	*Habenaria ovalifolium*	14	0.040801	182
16	*Habenaria rariflora*	24	0.062023	552
17	*Habenaria roxburghii*	59	0.119974	3422
18	*Luisia abrahami*	48	0.103671	2256
19	*Luisia macrantha*	125	0.196713	15500
20	*Luisia zeylanica*	14	0.040801	182
21	*Pholidoto pallida*	11	0.033682	110
22	*Polystachya concreta*	30	0.073429	870
23	*Rhyncostylis retusa*	39	0.089192	1482
24	*Vanda tessellata*	51	0.108257	2550
25	*Vanda testacea*	312	0.316236	97032
	TOTAL	1633	2.817341	221824

4.1.7 Comparison of orchid diversity between different habitats

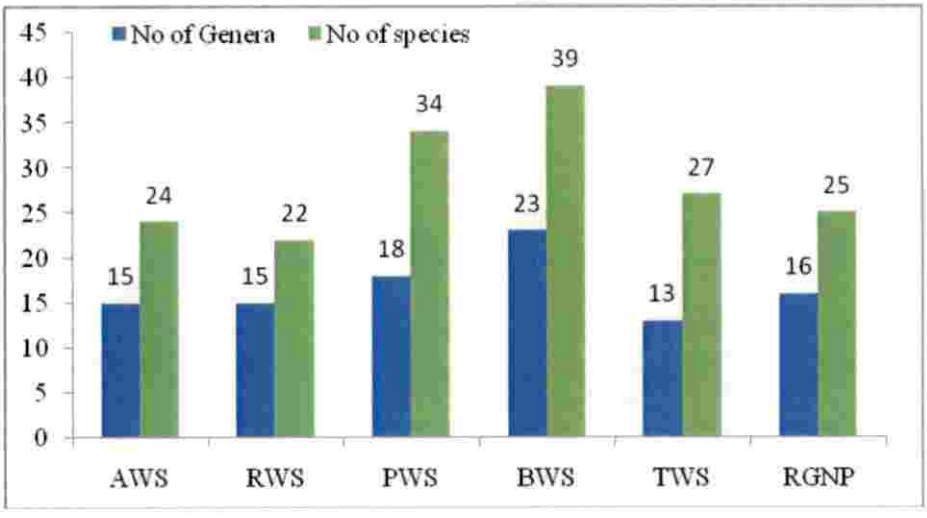
The maximum number of genera was found in the Brahmagiri Wildlife Sanctuary with a total of 23 genera comprising of 39 species. The minimum number of genera was noted in the Talakavery Wildlife Sanctuary with a total of 13 genera and the lowest number of species were recorded in Ranipuram Wildlife Sanctuary with a total of 22 species. (Figure 2).

The extent of diversity of orchids present in the 6 different habitats surveyed were estimated using Shannon-Simpson indices. The result shows that orchid diversity is more in Brahmagiri Wildlife Sanctuary (Shannon index = 3.37991) (Table 9).

Table 9. Extent of orchid diversity in Central Western Ghats based on Shannon and Simpson Index

Sl. No.	Habitat	Shannon index	Simpson index	Simpson index of diversity
1	Aralam Wildlife Sanctuary	2.984732	0.058436	0.94156
2	Ranipuram Wildlife Sanctuary	2.540636	0.124028	0.87597
3	Pushpagiri Wildlife Sanctuary	3.274464	0.048877	0.95112
4	Brahmagiri Wild life Sanctuary	3.379914	0.041657	0.95834
5	Talakavery Wildlife Sanctuary	3.019068	0.059632	0.94036
6	Rajeev Gandhi National Park	2.817342	0.083234	0.91676

Figure 2. Orchid genotypes in 6 different habitats of Central Western Ghats.



(AWS Aralam Wildlife Sanctuary,RWS- Ranipuram Wildlife Sanctuary -PWS-Pushpagiri Wildlife Sanctuary, BWS-Brahmagiri Wildlife Sanctuary ,TWS-Talakavery Wildlife Sanctuary, RGNP- Rajeev Gandhi National Park.).

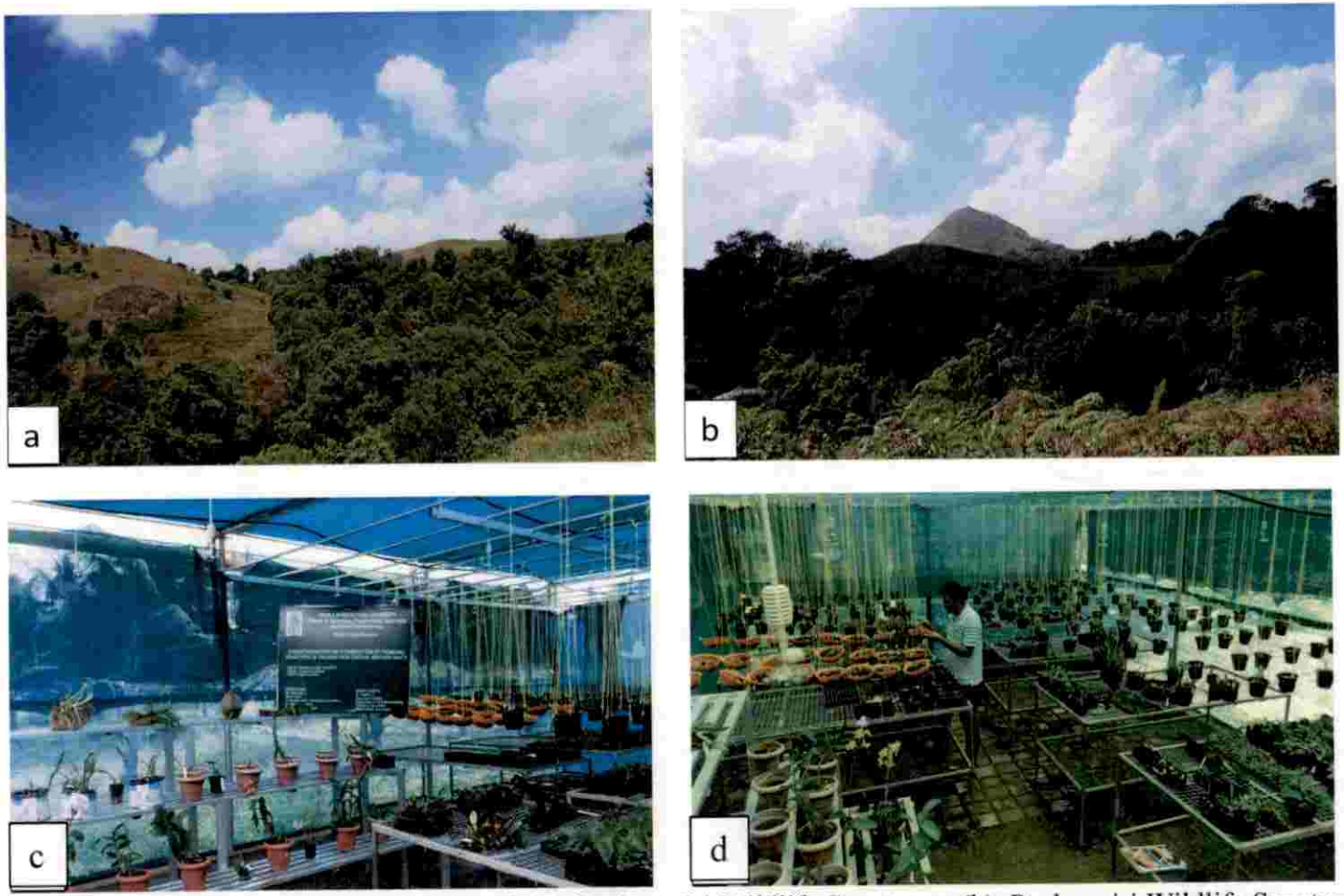


Plate 1: (a), (b): A panoramic view of Shola forest of, (a). Brahmagiri Wildlife Sanctuary, (b). Pushpagiri Wildlife Sanctuary; (c) (d): Inside view of orchidarium at College of Agriculture, Padannakkad.

4.2 CLIMATIC PARAMETERS RECORDED AT *IN SITU* AND *EX SITU* CONDITIONS

4.2.1 *In situ* conditions (Central Western Ghats)

Observations were recorded on the weather parameters during the survey in the 6 habitats where the orchids are growing under natural conditions. The parameters which were recorded comprise of the habitat, location, latitude, longitude, elevation in meters, light intensity in LUX, temperature at the habitat in degree celsius, canopy temperature in degree celsius, relative humidity in percentage, and wind speed in km/h (Table 10 & 11).

4.3 IDENTIFICATION OF ORCHID SPECIES AND ESTABLISHMENT IN ORCHIDARIUM

After the survey, some of the species were rescued and an orchidarium was established at CoA, Padannakkad. This includes 46 accessions consisting of wild types and hybrids obtained from different places as detailed in materials and methods (Table 1). These were further subjected to morphological and molecular characterization (Plate 1; Plate 4; Plate 5; Plate 6)

Table 10.Natural location of orchid genotypes rescued and maintained in orchidarium.

Accession code	Name of species	Location	Latitude (North)	Longitude (East)	Elevation (m) AMSL
PDK/ORP-1	*Vanda testacea*	Nagarhole	12°14.79'	76°3.48'	875
PDK/ORP-2	*Vanda*	Nagarhole	12°8.74'	75°56.28'	856
PDK/ORP-3	*Vanda*	Nagarhole	12°10.33'	75°59.22'	839
PDK/ORP-4	*Vanda*	Thithmathi	12°13.39'	76°0.06'	857
PDK/ORP-5	*Vanda*	Thithmathi	12°13.54'	76°0.80'	867
PDK/ORP-6	*Vanda*	Sikkim*	27°20.35'	88°36.40'	1509
PDK/ORP-7	*Cymbidium*	Sikkim*	27°20.35'	88°36.40'	1509
PDK/ORP-8	*Dendrobium*	Padannakkad*	12°15.42'	75°7.05'	14
PDK/ORP-9	*Coelogyne*	Sikkim*	27°20.35'	88°36.40'	1509
PDK/ORP-10	*Phalenopsis* hybrid	Bengaluru*	13°3.76'	77°37.88'	907
PDK/ORP-11	*Dendrobium* hybrid 1	Bengaluru*	13°3.76'	77°37.88'	907

PDK/ORP-12	*Oncidium tolumina*	Bengaluru*	13°3.76'	77°37.88'	907
PDK/ORP-13	*Dendrobium* hybrid 2	Bengaluru*	13°3.76'	77°37.88'	907
PDK/ORP-14	Mini *Cattleya*	Bengaluru*	13°3.76'	77°37.88'	907
PDK/ORP-15	*Dendrobium* hybrid 3	Bengaluru*	13°3.76'	77°37.88'	907
PDK/ORP-16	*Dendrobium* hybrid 4	Bengaluru*	13°3.76'	77°37.88'	907
PDK/ORP-17	*Dendrobium* hybrid 5	Bengaluru*	13°3.76'	77°37.88'	907
PDK/ORP-18	*Dendrobium* hybrid 6	Bengaluru*	13°3.76'	77°37.88'	907
PDK/ORP-19	*Vanda*	Sullia	12°29.99'	75°23.61'	301
PDK/ORP-20	*Dendrobium*	Bharamagiri	11°55.34'	75°59.38'	1576
PDK/ORP-21	*Vanda*	Nagarhole	12°7.54'	76°9.67'	867
PDK/ORP-22	*Cattleya*	Gonnikoppa	12°9.30'	75°53.36'	827
PDK/ORP-23	*Vanda*	Thithmathi	12°13.00'	76°5.39'	838
PDK/ORP-24	*Cymbidium*	Sikkim*	27°20.35'	88°36.40'	1509
PDK/ORP-25	*Cymbidium*	Sikkim*	27°20.35'	88°36.40'	1509
PDK/ORP-26	*Cymbidium*	Sikkim*	27°20.35'	88°36.40'	1509
PDK/ORP-27	*Cymbidium*	Sikkim*	27°20.35'	88°36.40'	1509
PDK/ORP-28	*Coelogyne breviscapa*	Thithmathi	12°13.00'	76°5.39'	838
PDK/ORP-29	*Bulbophyllum elongatum*	Thithmathi	12°13.61'	75°59.49'	824
PDK/ORP-30	*Aerides maculosa*	Mayamudi	12°12.97'	75°59.33'	839
PDK/ORP-31	*Bulbophyllum. fischeri*	Bharamagiri	11°58.87'	75°56.62'	1086
PDK/ORP-32	*Pholidota imbricate*	Bharamagiri	11°54.81'	75°51.24'	898
PDK/ORP-33	*Rhynchostylis retusa*	Bharamagiri	11°54.81'	75°51.24'	1015
PDK/ORP-34	*Dendrobium aqueum*	Bharamagiri	11°58.87'	75°56.62'	1320
PDK/ORP-35	*Cymbidium*	Pushpagiri	12°39.55'	75°38.06'	309
PDK/ORP-36	*Vanda*	Bharamagiri	11°54.81'	75°51.24'	946
PDK/ORP-37	*Rhynchostylis retusa*	Ponnampet	12°8.69'	75°56.24'	855
PDK/ORP-38	*Phalaenopsis* (T.C)	Padannakkad*	12°15.42'	75°7.05'	14
PDK/ORP-39	*Flickingeria nodosa*	Pushpagiri	12°14.79'	76°3.48'	1158
PDK/ORP-40	*Vanda*	Pushpagiri	12°8.74'	75°56.28'	1132
PDK/ORP-41	*Vanda*	Pushpagiri	12°10.33'	75°59.22'	1037
PDK/ORP-42	*Dendrobium* (T.C)	Padannakkad*	12°13.39'	76°0.06'	15
PDK/ORP-43	*Vanda*	Nagarhole	12°13.54'	76°0.80'	866
PDK/ORP-44	*Vanda*	Nagarhole	12°13.54'	76°0.80'	866
PDK/ORP-45	*Acampe praemorsa*	Padannakkad*	12°13.39'	76°0.06'	15
PDK/ORP-46	*Vanda*	Nagarhole	12°15.42'	75°7.05'	839

(AMSL- Above Mean Sea Level; T.C- Tissue culture plant; * collected from places other than central western ghats)

Table 11. *In situ* microclimatic parameters in Central Western Ghats during survey for orchid diversity

Accession code	Name of species	Light intensity (Lux)	Temp. in habitat (^{0} C)	Temp. in canopy (^{0} C)	RH (%)	Wind speed (Km/h)
PDK/ORP-1	*Vanda testacea*	154	16	12	60	12
PDK/ORP-2	*Vanda*	526.2	12.8	11.5	60	12
PDK/ORP-3	*Vanda*	342	18	16	60	12
PDK/ORP-4	*Vanda*	806.5	19.2	18.6	58	8
PDK/ORP-5	*Vanda*	116.2	16	14.83	52	6
PDK/ORP-6	*Vanda*	*	*	*	83	9
PDK/ORP-7	*Cymbidium*	*	*	*	83	9
PDK/ORP-8	*Dendrobium*	1506	26.5	26	90	10
PDK/ORP-9	*Coelogyne*	*	*	*	83	9
PDK/ORP-10	*Phalenopsis* hybrid	283	28	28	36	10
PDK/ORP-11	*Dendrobium* hybrid 1	283	28	28	36	10
PDK/ORP-12	*Oncidium tolumina*	254	28	28	36	10
PDK/ORP-13	*Dendrobium* hybrid 2	208	28	28	36	10
PDK/ORP-14	Mini *Cattleya*	305	28	28	36	10
PDK/ORP-15	*Dendrobium* hybrid 3	305	28	28	36	10
PDK/ORP-16	*Dendrobium* hybrid 4	449	28	28	36	10
PDK/ORP-17	*Dendrobium* hybrid 5	729	28	28	36	10
PDK/ORP-18	*Dendrobium* hybrid 6	281	28	28	36	10
PDK/ORP-19	*Vanda*	146.2	14	15.3	28	3
PDK/ORP-20	*Dendrobium*	92	11.8	11	86	12
PDK/ORP-21	*Vanda*	281	16.9	15.2	40	16
PDK/ORP-22	*Cattleya*	121	22.3	20.16	60	10
PDK/ORP-23	*Vanda*	506.1	18.4	18.3	52	11
PDK/ORP-24	*Cymbidium*	*	*	*	83	9
PDK/ORP-25	*Cymbidium*	*	*	*	83	9
PDK/ORP-26	*Cymbidium*	*	*	*	83	9
PDK/ORP-27	*Cymbidium*	*	*	*	83	9
PDK/ORP-28	*Coelogyne breviscapa*	350.2	18.4	17.5	60	11
PDK/ORP-29	*Bulb. Elongatum*	169.3	16.2	14	60	9
PDK/ORP-30	*Aerides maculosa*	111.98	15.36	15.02	59	9
PDK/ORP-31	*Bulb. Fischeri*	56.36	11.63	11.12	70	12
PDK/ORP-32	*Pholidota imbricate*	127.3	11.8	10.8	70	12
PDK/ORP-33	*Rhynchostylis retusa*	258	10.13	9	70	12
PDK/ORP-34	*Den. Aqueum*	45.6	16.3	11.23	62	16

PDK/ORP-35	*Cymbidium*	89.6	12.3	12.3	62	16
PDK/ORP-36	*Vanda*	120.7	19.2	18.6	62	16
PDK/ORP-37	*Rhynchostylis retusa*	356	20.13	19.8	60	10
PDK/ORP-38	*Phalaenopsis (T.C)*	1458	28.3	27.03	94	2
PDK/ORP-39	*Flickingeria nodosa*	782.1	10.25	8.2	52	14
PDK/ORP-40	*Vanda*	245.6	13	9.63	52	14
PDK/ORP-41	*Vanda*	80.18	10.34	10.21	52	14
PDK/ORP-42	*Dendrobium (T.C)*	161	28.3	27	92	1
PDK/ORP-43	*Vanda*	161	19.2	18.68	70	12
PDK/ORP-44	*Vanda*	387	24.63	23.33	58	8
PDK/ORP-45	*Acampe praemorsa*	326.5	28.3	28	92	1
PDK/ORP-46	*Vanda*	74.1	21.13	21.3	58	16

(Temp.-temperature, RH-Relative Humidity; *- microclimatic parameters unavailable)

4.2.2 *Ex situ* conditions (College of Agriculture, Padannakkad)

Since the 46 accessions are maintained in an orchidarium at CoA, Padannakkad, the weather data obtained from Department of Meteorology, Regional Agricultural Research Station (RARS), Pilicode which is located nearby (10km from CoA, Padannakkad) representing the northern zone of Kerala were utilized for the study (Table 12). The exact location is N12^{0}12' Latitude and E75^{0}10' Longitude. The place is only 15 m above Mean Sea Level (AMSL).

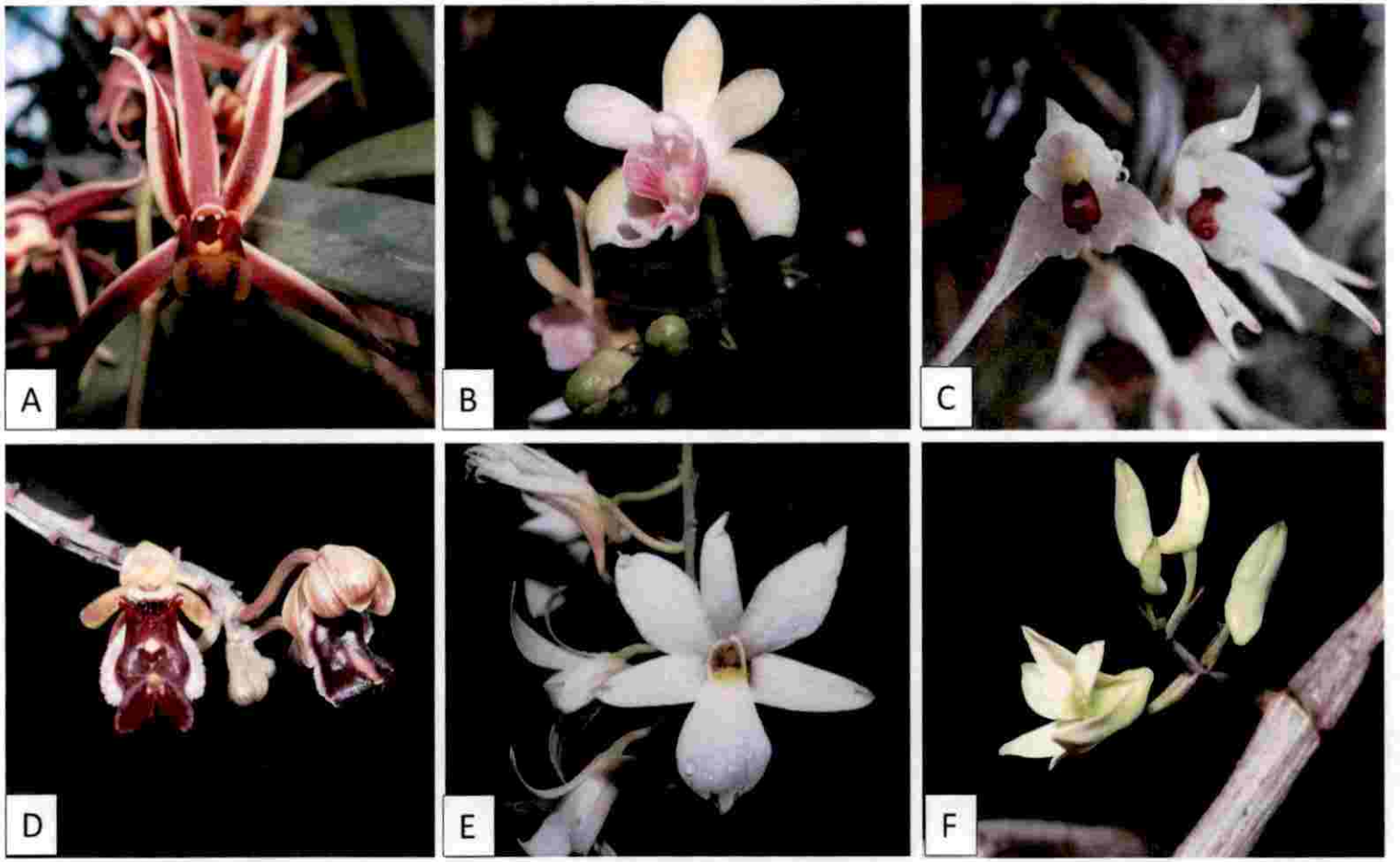


Plate 2: Orchids flowering in natural habitat: (A) *Cymbidium bicolour*, (B) *Deplocentrum congestum*, (C) *Bulbophyllum mysorense*, (D) *Cottonia pedunculairs*, (E) *Dendrobium barbatalum*, (F) *Dendrobium macrostachyum*.

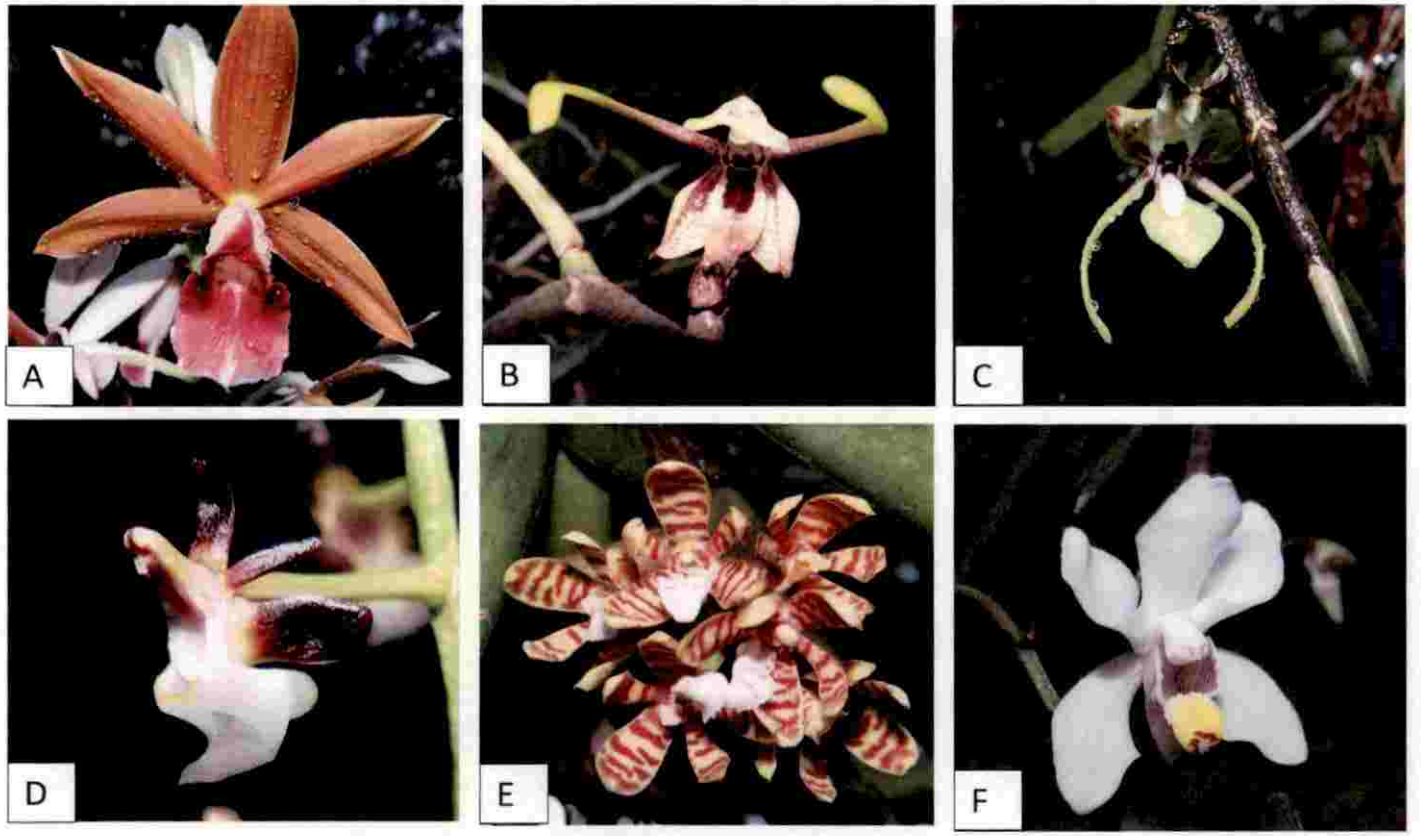


Plate 3: Orchids flowering in natural habitat: (A) *Phaius tankervilleae*, (B) (C), *Luisea macrantha*, (D) *Smithsonia sp*, (E) *Acampe praemorsa*, (F) *Papilionathe subultal*.

Table 12. Weather parameters near orchidarium at College of Agriculture, Padannakkad (January, 2014–June 2016).

Month & year	Max. Temp. (^{0} C)	Min. Temp (^{0} C)	Max. RH (%)	Min. RH (%)	Wind speed (Km/h)	Daily Av. Rainfall (mm)
Jan,14	32.59	20.30	91.68	58.81	1.90	0.00
Feb,14	32.92	21.36	91.75	61.21	2.41	0.00
March,14	33.24	22.19	86.87	61.42	2.66	0.00
Apr,14	34.41	25.06	82.47	65.23	2.85	0.03
May,14	33.42	23.97	85.19	68.87	2.43	4.64
June,14	31.73	23.73	89.43	76.20	1.86	19.78
July,14	29.74	22.87	91.77	82.26	1.10	30.18
Aug,14	28.92	22.94	93.90	82.61	0.72	37.65
Sept,14	30.29	23.06	91.90	73.03	1.68	11.74
Oct,14	31.36	23.57	89.74	70.23	1.20	5.98
Nov,14	32.42	21.98	87.80	65.87	1.12	1.25
Dec,14	32.00	21.40	89.80	60.60	1.00	1.90
Jan,15	31.66	19.34	89.68	57.90	1.61	0.00
Feb,15	32.56	19.98	91.32	58.21	2.02	0.00
March,15	32.58	23.51	88.26	62.13	3.26	0.00
Apr,15	33.03	23.98	84.23	63.93	2.29	58.00
May,15	32.62	23.94	87.26	68.81	1.81	126.10
June,15	30.76	22.75	91.03	79.03	2.27	532.60
July,15	30.01	23.45	95.35	80.77	1.11	29.13
Aug,15	30.35	23.41	93.74	80.74	1.28	17.62
Sept,15	31.24	23.44	93.33	82.53	1.49	12.34
Oct,15	31.35	23.62	92.84	76.30	1.68	8.57
Nov,15	31.39	23.05	91.37	72.13	1.20	3.56
Dec,15	32.26	21.82	94.19	68.35	1.32	0.12
Jan,16,	32.25	19.57	93.42	56.58	1.45	0.00
Feb,16	32.26	22.12	92.14	59.17	2.01	0.00
March,16	33.59	24.58	88.29	61.06	2.33	0.00
Apr,16	34.11	26.09	84.23	63.77	2.65	0.00
May,16	33.93	25.11	86.74	64.45	2.63	1.87
June,16	29.73	23.43	94.00	78.33	1.42	31.24

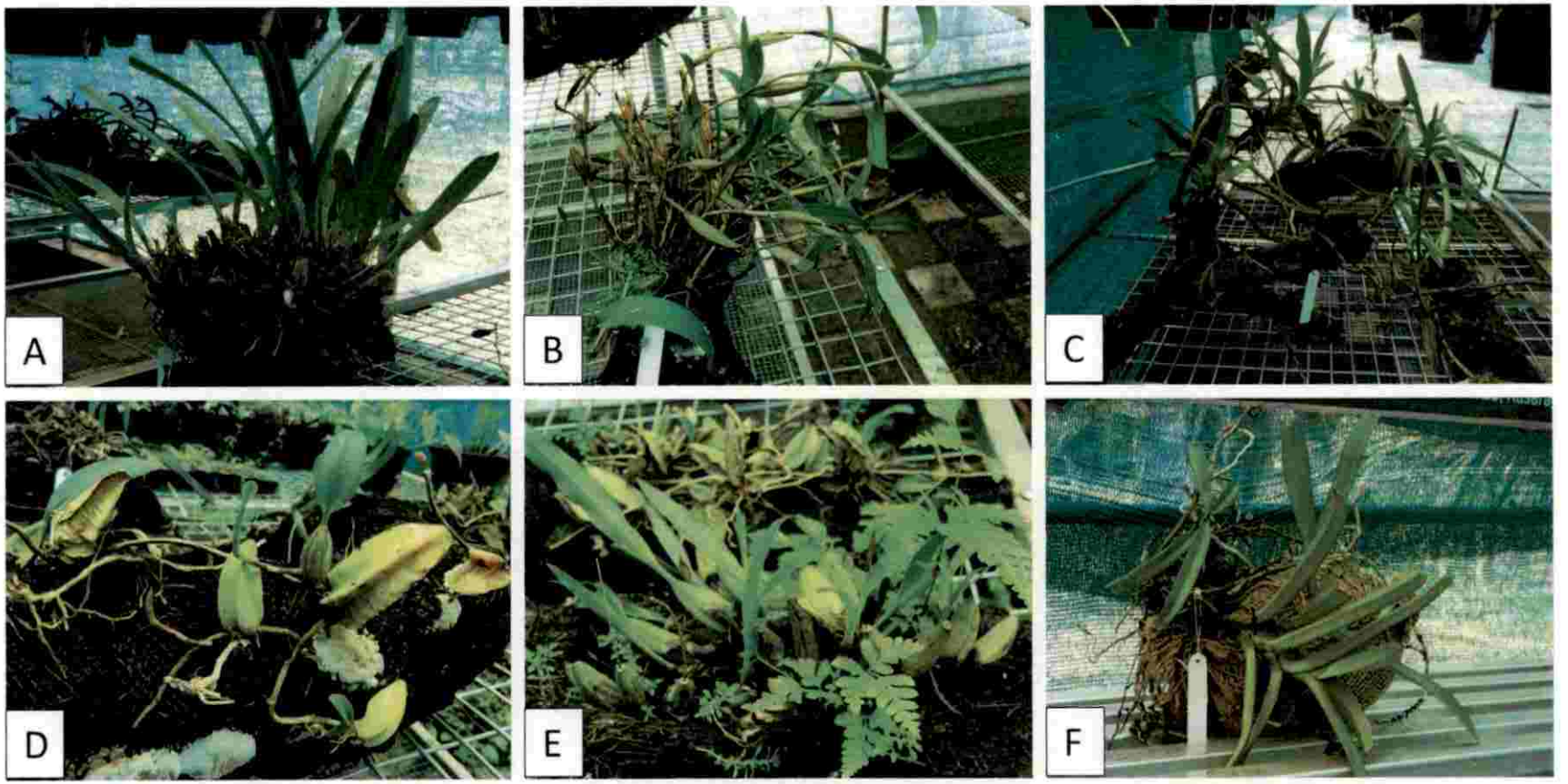


Plate 4: Orchid accessions present in orchidarium: (A) PDK/ORP-35, (B) PDK/ORP-39, (C) PDK/ORP-01, (D) PDK/ORP-29, (E) PDK/ORP-32, (F) PDK/ORP-43.

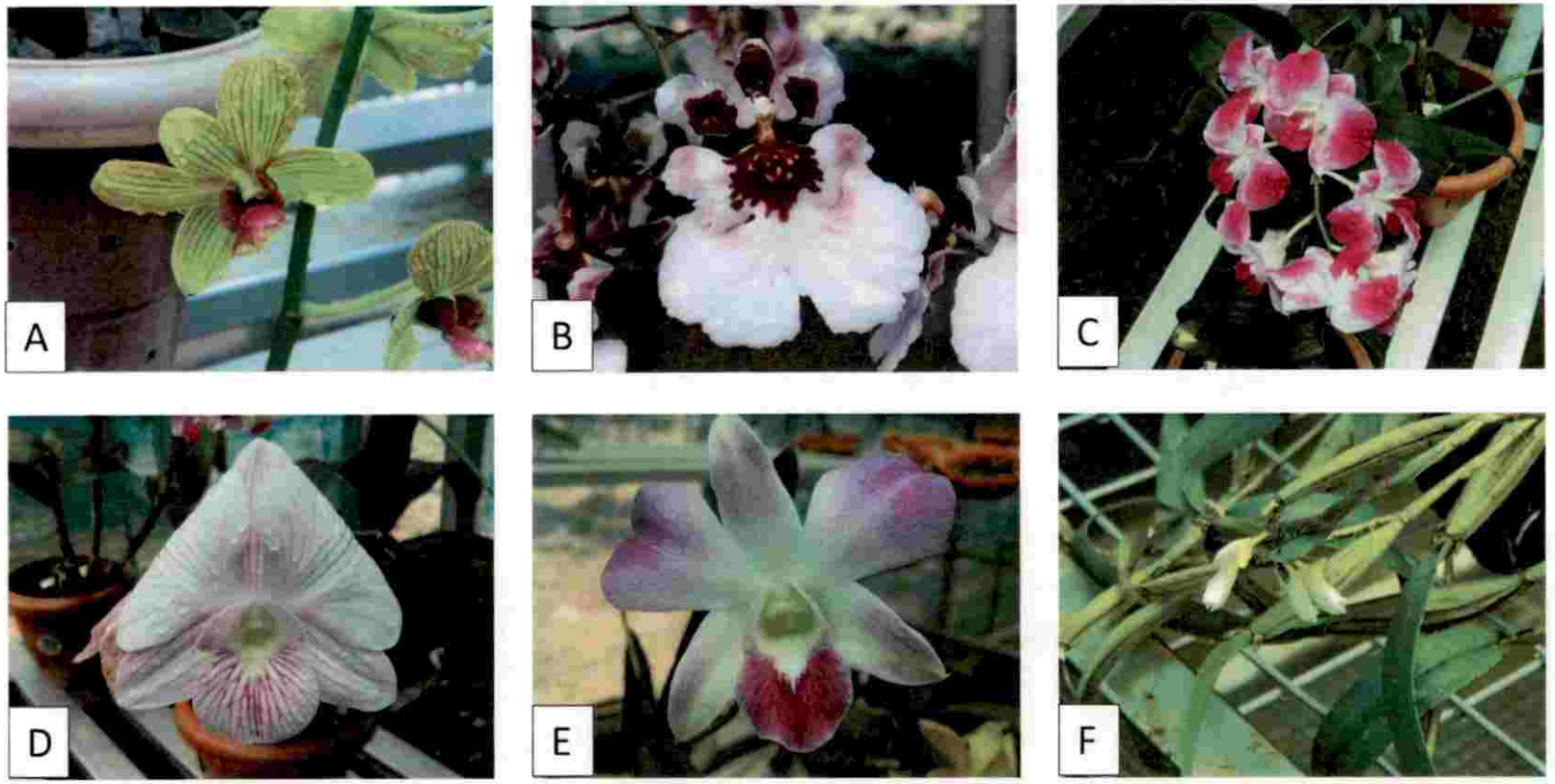


Plate 5: Accessions at the flowering stage in orchidarium: (A) PDK/ORP-11, (B) PDK/ORP-12, (C) PDK/ORP-16, (D) PDK/ORP-17, (E) PDK/ORP-20, (F) PDK/ORP-39.

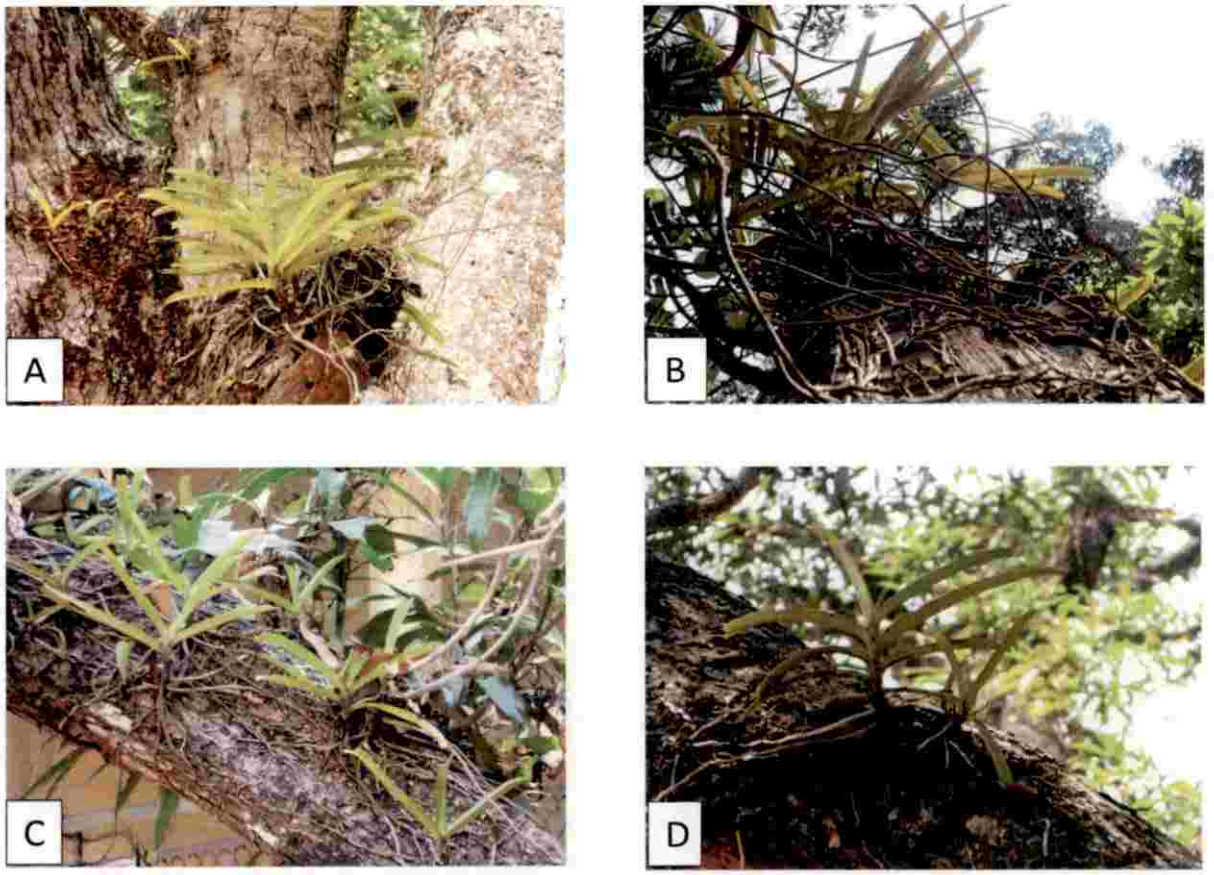


Plate 6: *Ex situ* conservation of orchids in CoA, Padannakkad (A) T_1-Tree 1, (B) T_2-Tree 2, (C) T_3-Tree 3, (D) T_4-Tree 4.

4.4 MORPHOLOGICAL CHARACTERISATION

Morphological characters of the 46 accessions in orchidarium were recorded based on the descriptor provided by National Research Centre for Orchids, Sikkim (NRCO). Out of the total 46 genotypes established in the orchidarium at CoA Padannakkad, only 41 accessions survived during the later stage of the study. The 5 accessions which did not get established under the changed agro-climatic conditions include 2 *Cymbidium* species (PDK/ORP-26, PDK/ORP-27) from Sikkim, 1 *Rhynchostylis retusa* (PDK/ORP-37) from Ponnampet and 2 *Vanda* species (PDK/ORP-40, PDK/ORP-41) from Pushpagiri Wildlife Sanctuary.

4.4.1 Vegetative characters

Vegetative characters recorded include qualitative (descriptive) traits such as nature of pseudo bulb, shape of leaf and leaf apex as well as quantitative traits such as plant height, leaf length and width, number of leaves, intermodal length and number of sprouts.

4.4.1.1 Vanda spp.

Out of the 14 genera, *Vanda* had the largest number of accessions (13nos out of 15 survived) which varied in their vegetative characteristics. Except for the accession PDK/ORP-3, all the 13 species had woody stem and strapped leaf shape while accession PDK/ORP-3 has channeled leaf shape. Leaf apex, however, showed variation with 6 bilobed, 5 retuse and 2 truncated leaf apices (Table 13) (Plate 7; Plate 8).

With respect to the quantitative traits, among these 13 *Vanda* species accession number PDK/ORP-5 was the tallest with plant height 126 cm and accession number PDK/ORP-6 was the smallest with plant height 5.9 cm (Table 14). [1]Longest leaf was observed in PDK/ORP-3 (37.9cm) and smallest in PDK/ORP-6 (5cm). Out of these 13 *Vanda* species, highest and lowest leaf width

[1] As per the descriptor for the plant height accessions were classified as very large (2 nos), large (2 nos), medium (3 nos), small (5 nos) and vey small (1 nos).

was observed in PDK/ORP-19 (3.4cm) and PDK/ORP-6 (0.9cm) respectively; PDK/ORP-19 (4.0cm) and PDK/ORP-6 (nil) had highest and lowest internodal length respectively. The total number of leaves was observed highest in PDK/ORP-5 (41nos) and lowest in PDK/ORP-46 (4nos); whereas the number of sprouts was highest in PDK/ORP-2 (6) and no sprouts were observed in the PDK/ORP-19, PDK/ORP-44, and PDK/ORP-46 (Table 14).

Table 13: Descriptive traits (vegetative plant parts) recorded for the orchid accessions maintained in orchidarium.

Sl No.	Genotype	Accession code	Nature of pseudo bulb	Shape of leaf	Leaf apex
1.	*Acampe praemorsa*	PDK/ORP-45	Woody	Strap	Bilobed
2.	*Aeridis maculosa*	PDK/ORP-30	Woody	Strap	Bilobed
3.	*Bulbophyllum elongatum*	PDK/ORP-29	Bulbous	Elliptic	Obtuse
4.	*Bulbophyllum fischeri*	PDK/ORP-31	Bulbous	Elliptic	Obtuse
5.	*Cattleya*	PDK/ORP-22	Cylindrical	Ligulate	Bilobed
6.	*Coelogyne*	PDK/ORP-9	Fleshy	Oblong	Acute
7.	*Coelogyne breviscapa*	PDK/ORP-28	Clavate fleshy	Linear oblong	Acute
8.	*Cymbidium*	PDK/ORP-7	Ovoid	Linear	Acute
9.	*Cymbidium*	PDK/ORP-24	Conical	Linear	Acute
10.	*Cymbidium*	PDK/ORP-25	Conical	Linear	Acute
11.	*Cymbidium*	PDK/ORP-26	Conical	Linear	Acute
12.	*Cymbidium*	PDK/ORP-27	Ovoid	Linear	Acute
13.	*Cymbidium*	PDK/ORP-35	Ovoid	Linear oblong	Bilobed
14.	*Dendrobium*	PDK/ORP-8	Fleshy	Lanceolate	Acute
15.	*Dendrobium*	PDK/ORP-20	Cane fleshy	Lanceolate	Obtuse
16.	*Dendrobium* (T.C)	PDK/ORP-42	Clavate fleshy	Lanceolate	Obtuse
17.	*Dendrobium aqueum*	PDK/ORP-34	Woody	Lanceolate	Acute
18.	*Dendrobium* hybrid 1	PDK/ORP-11	Clavate fleshy	Lanceolate	Acute
19.	*Dendrobium* hybrid 2	PDK/ORP-13	Clavate fleshy	Lanceolate	Acute
20.	*Dendrobium*	PDK/ORP-15	Clavate fleshy	Elliptic	Retuse

	hybrid 3				
21.	*Dendrobium* hybrid 4	PDK/ORP-16	Clavate fleshy	Elliptic	Acute
22.	*Dendrobium* hybrid 5	PDK/ORP-17	Clavate fleshy	Lanceolate	Retuse
23.	*Dendrobium* hybrid 6	PDK/ORP-18	Clavate fleshy	Elliptic	Retuse
24.	*Flickingeria nodosa*	PDK/ORP-39	Clavate	Narrow oblong	Acute
25.	Mini *Cattleya*	PDK/ORP-14	Ovoid	Elliptic	Obtuse
26.	*Oncidium tolumina*	PDK/ORP-12	Fleshy	Linear	Acute
27.	*Phalaenopsis* (T.C)	PDK/ORP-38	Fleshy	Oblong	Acute
28.	*Phalenopsis hybrid*	PDK/ORP-10	Fleshy	Oblong	Acute
29.	*Pholidota imbricata*	PDK/ORP-32	Bulbous	Lanceolate	Acute
30.	*Rhynchostylis retusa*	PDK/ORP-33	Woody	Strap	Bilobed
31.	*Rhynchostylis retusa*	PDK/ORP-37	Woody	Strap	Bilobed
32.	*Vanda testacea*	PDK/ORP-1	Woody	Strap	Truncate
33.	*Vanda*	PDK/ORP-2	Woody	Strap	Retuse
34.	*Vanda*	PDK/ORP-3	Woody	Channeled	Bilobed
35.	*Vanda*	PDK/ORP-4	Woody	Strap	Retuse
36.	*Vanda*	PDK/ORP-5	Woody	Strap	Retuse
37.	*Vanda*	PDK/ORP-6	Woody	Strap	Retuse
38.	*Vanda*	PDK/ORP-19	Woody	Strap	Retuse
39.	*Vanda*	PDK/ORP-21	Woody	Strap	Truncate
40.	*Vanda*	PDK/ORP-23	Woody	Strap	Bilobed
41.	*Vanda*	PDK/ORP-36	Woody	Strap	Bilobed
42.	*Vanda*	PDK/ORP-40	Woody	Strap	Bilobed
43.	*Vanda*	PDK/ORP-41	Woody	Strap	Truncate
44.	*Vanda*	PDK/ORP-43	Woody	Strap	Bilobed
45.	*Vanda*	PDK/ORP-44	Woody	Strap	Bilobed
46.	*Vanda*	PDK/ORP-46	Woody	Strap	Bilobed

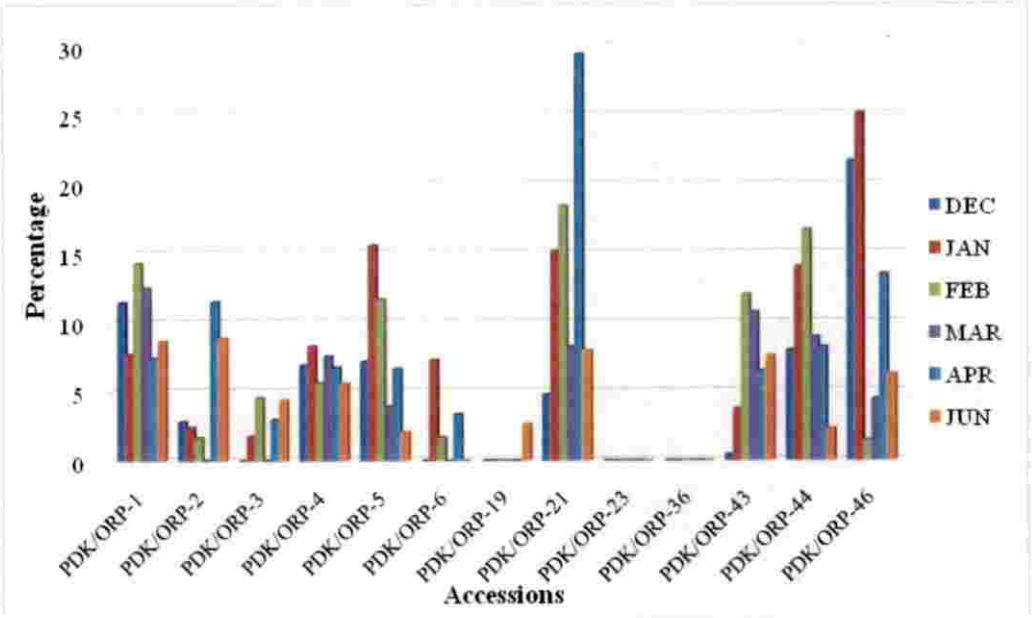


Figure 3. Absolute growth in plant height among the different *Vanda* species during 2015-16

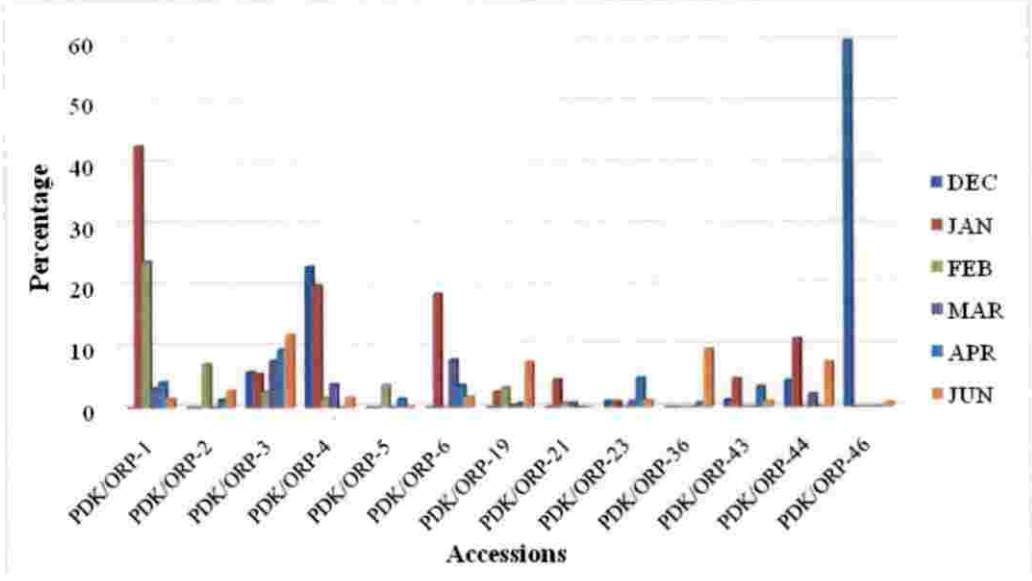


Figure 4. Absolute growth in leaf length among the different *Vanda* species during 2015-16

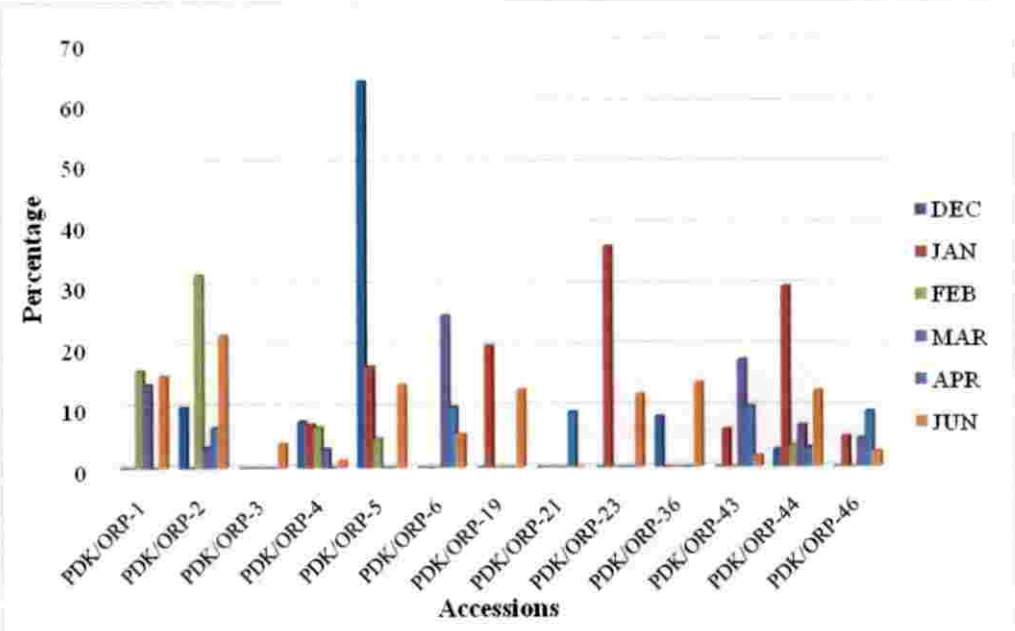


Figure 5. Absolute growth in leaf width among the different *Vanda* species during 2015-16

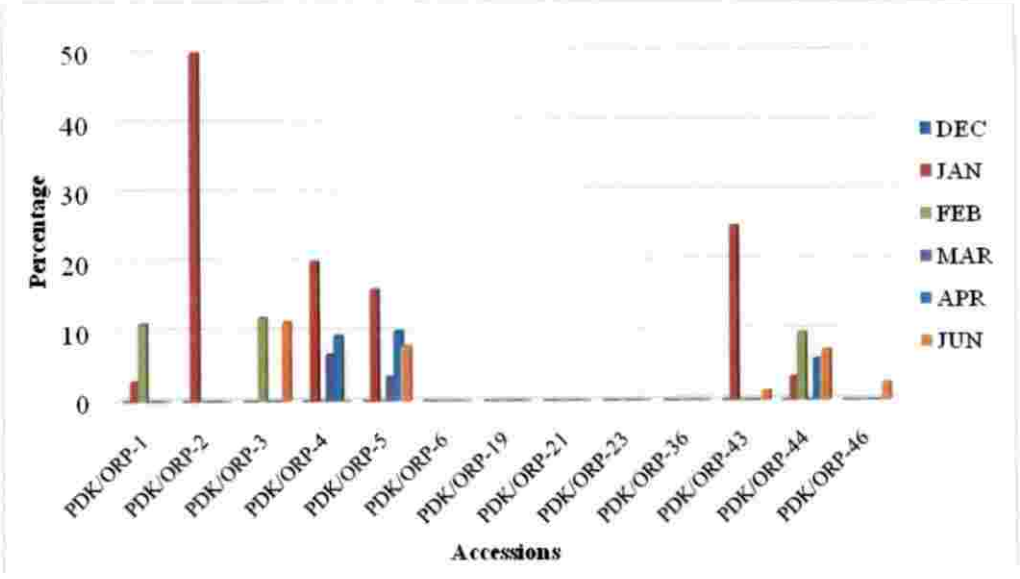


Figure 6. Absolute growth in internodal length among the different *Vanda* species during 2015-16

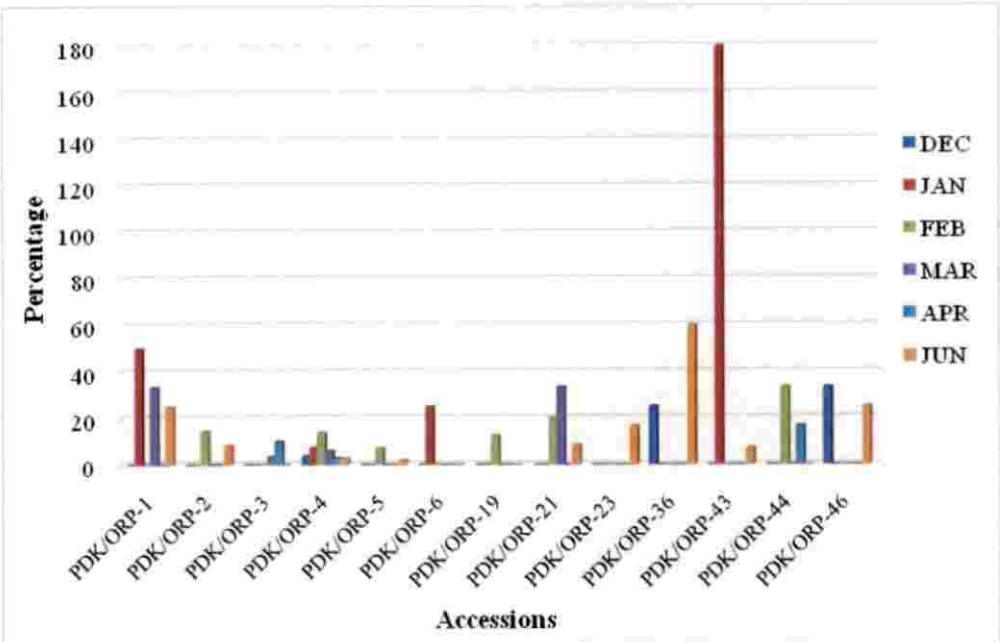


Figure 7. Absolute growth in number of leaves among the different *Vanda* species during 2015-16

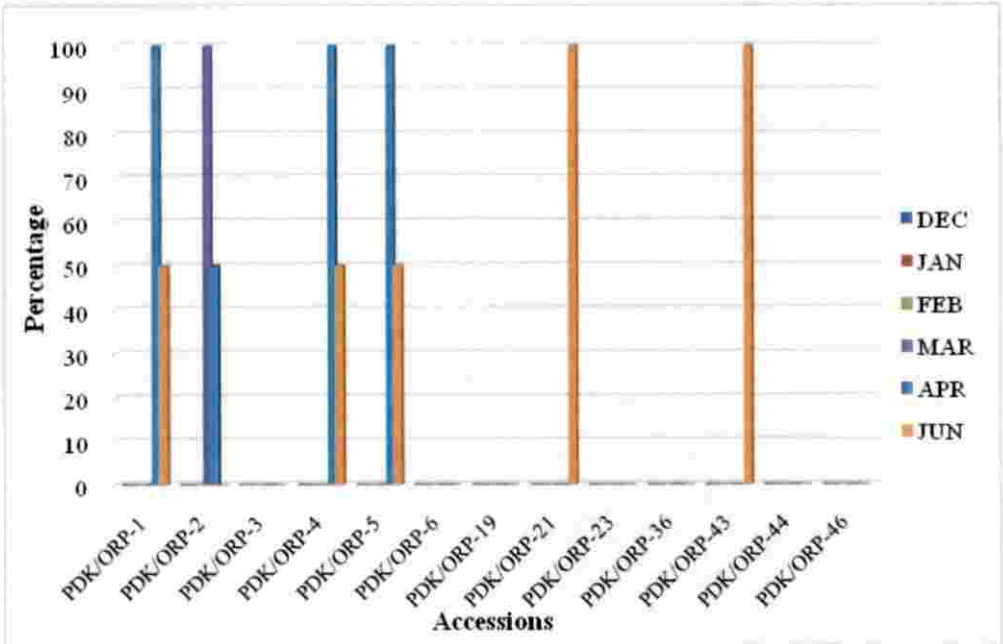


Figure 8. Absolute growth in number of sprouts among the different *Vanda* species during 2015-16

The absolute growth rate were recorded in percentage and highest absolute growth in plant height was observed in PDK/ORP-21 in April (29%). Absolute growth for leaf length was recorded as highest in PDK/ORP-46 (60%), and that of leaf width was highest in PDK/ORP-5 (64%) in December. Internodal length recorded highest absolute growth in PDK/ORP-2 in January (50%) while the highest absolute growth for number of leaves was for PDK/ORP-43 in January (180%). Absolute growth in number of sprouts was observed highest in PDK/ORP-2 in March (100%), in April (100%) for PDK/ORP-1,4,5 and in June (100%) for PDK/ORP-21and 43 (Fig. 3 – 8).

4.4.1.2 Dendrobium spp.

Dendrobium was the second large group with 10 accessions, which varied in their vegetative characters. Among these 10 accessions, 8 were with clavate fleshy stem while for the remaining two, one was with fleshy and one with woody stem. Leaf shape showed variation with 7 having lanceolate shape while 3 were with elliptic shape. Leaf apex varied with 5 acute, 3 retuse and 2 obtuse in shape (Table 14) (Plate 7; Plate 8).

PDK/ORP-13 was the tallest with plant height 51.4 cm among these and the smallest was PDK/ORP-8 with plant height 13.3 cm; the highest and lowest leaf length was measured in PDK/ORP-18 (17cm) and PDK/ORP-16 (6.6cm) respectively. The leaf width was observed highest in PDK/ORP-15(6.5cm) and lowest in PDK/ORP-34 (1.3cm); the highest and lowest internodal length was measured in PDK/ORP-11 (4.0cm) and PDK/ORP-18 (nil) respectively and the total number of leaves was highest and lowest in accession number PDK/ORP-42 (19nos) and PDK/ORP-20 (3nos) respectively. The number of sprouts was observed highest in PDK/ORP-42 (12 nos) whereas and sprout absent in PDK/ORP-11. Further the value obtained are classified as small, medium, large, long, narrow, broad, few, and many as per the *Dendrobium* descriptor (Table 14).

The absolute growth rate were recorded in percentage and highest absolute growth in plant height was observed in PDK/ORP-8 in December (51%). Absolute growth for leaf length was recorded as highest in PDK/ORP-42 in June (24%) and that of leaf width was highest in PDK/ORP-34 in April (25%). Internodal length recorded highest absolute growth in PDK/ORP-8 in February (85%) while the highest absolute growth for number of leaves was for PDK/ORP-34 in December (300%). Absolute growth in number of sprouts was observed highest in PDK/ORP-13, 15, 16, 17, 20 and 42 in June (100%) (Fig. 9-14).

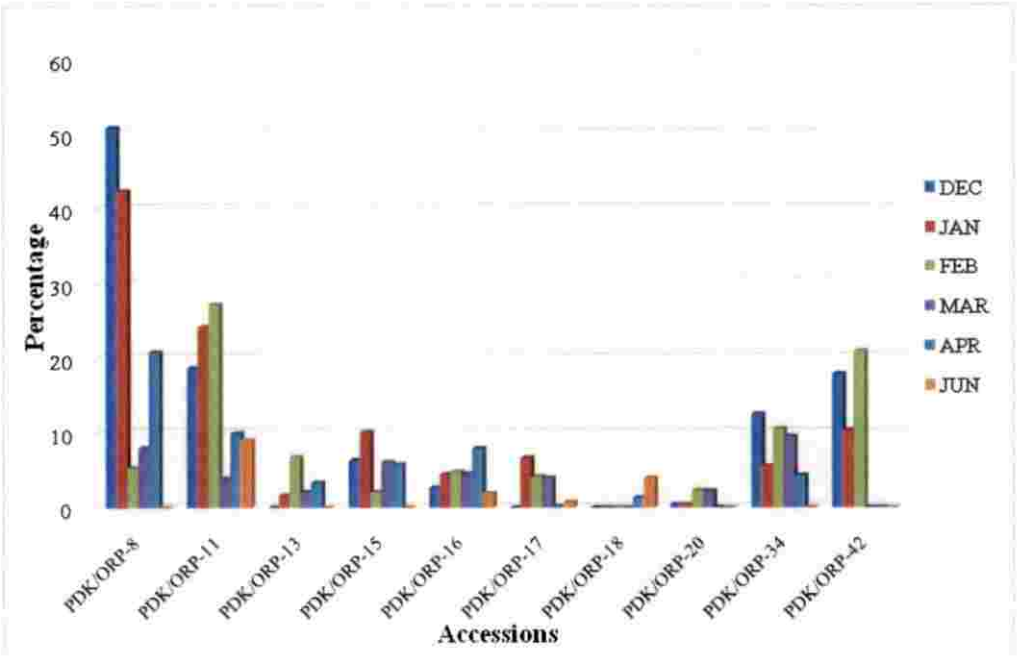


Figure 9. Absolute growth in plant height among the different *Dendrobium* species during 2015-16

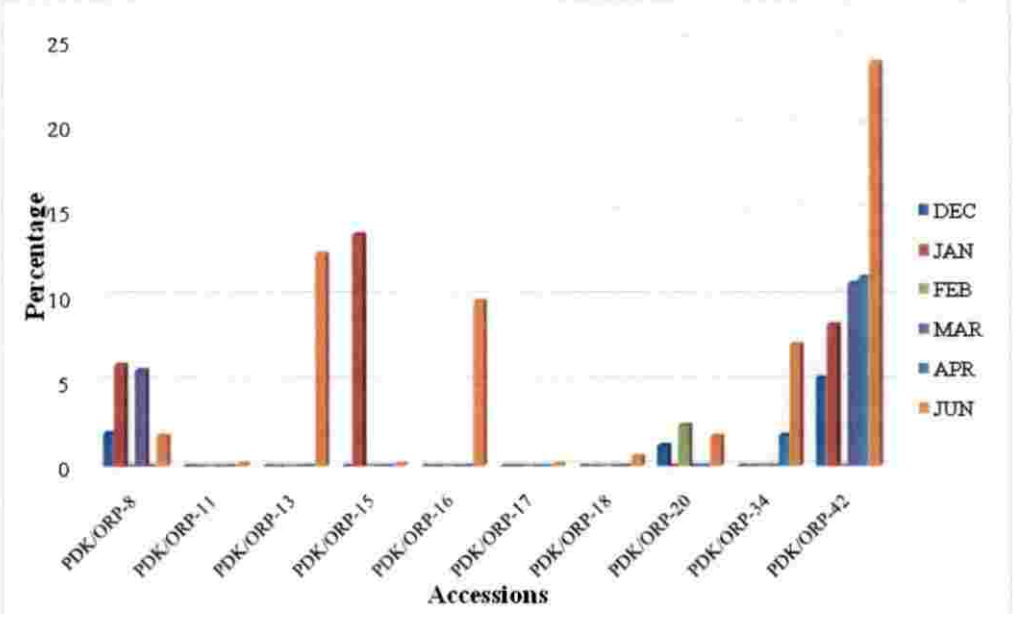


Figure 10. Absolute growth in leaf length among the different *Dendrobium* species during 2015-16

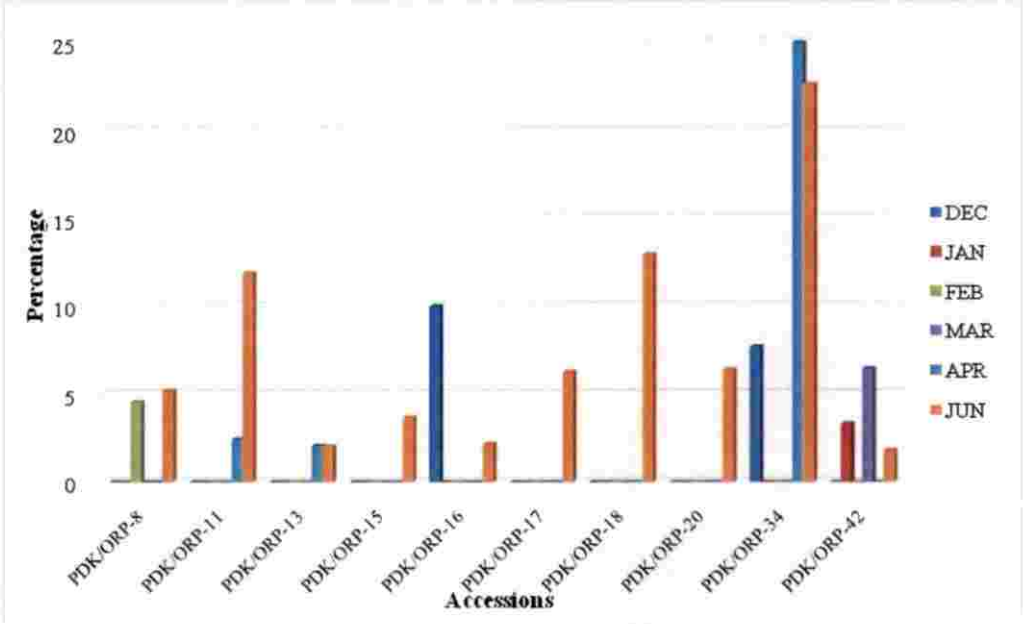


Figure 11. Absolute growth in leaf width among the different *Dendrobium* species during 2015-16

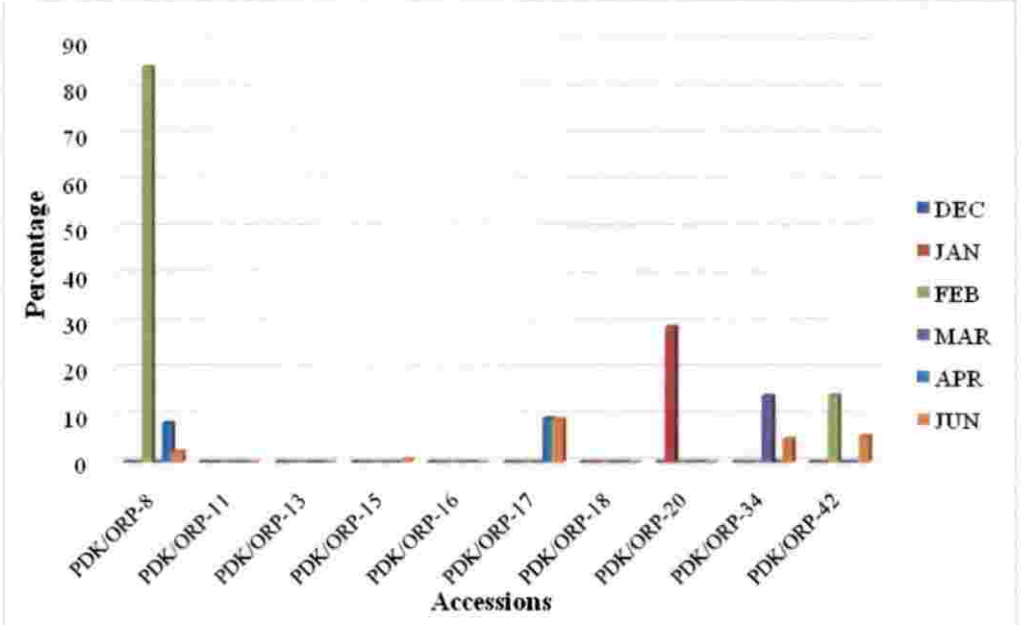


Figure 12. Absolute growth in internodal length among the different *Dendrobium* species during 2015-16

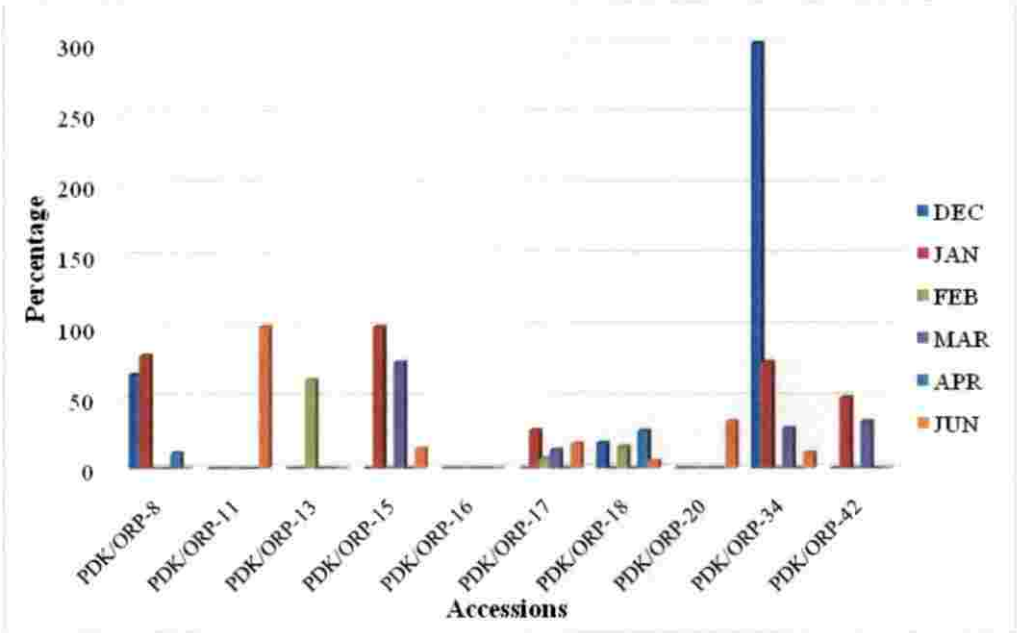


Figure 13. Absolute growth in number of leaves among the different *Dendrobium* species during 2015-16

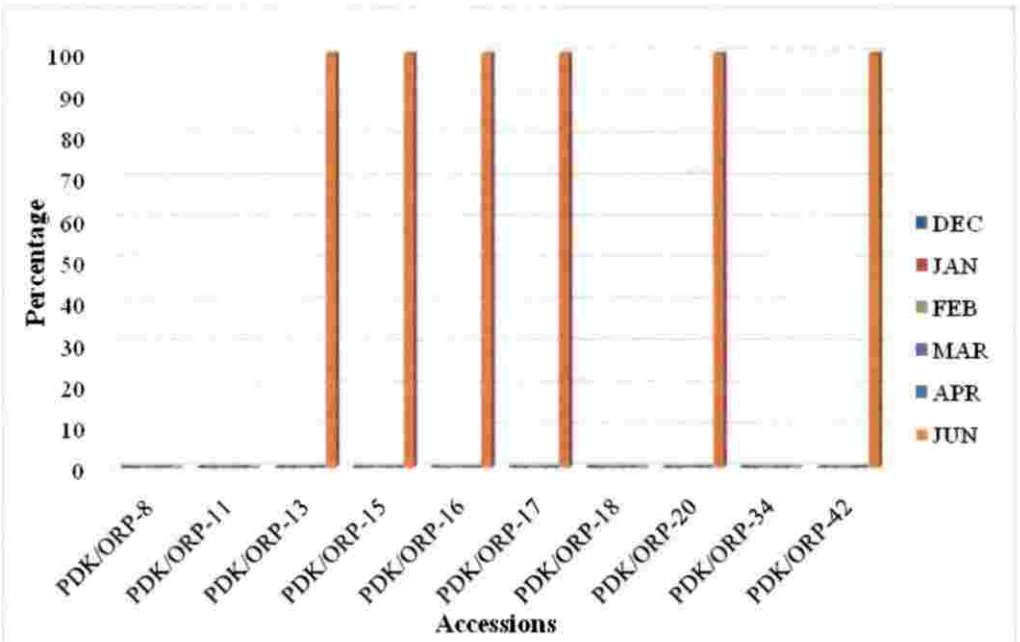


Figure 14. Absolute growth in number of sprouts among the different *Dendrobium* species during 2015-16

4.4.1.3 Cymbidium spp.

Out of six accessions under genus *Cymbidium*, four accessions survived, of which 2 had ovoid and 2 had conical stem; the leaf shape of 3 was linear whereas remaining one had linear-oblong shaped leaves; similarly 3 had acute leaf apex whereas remaining one had bilobed leaf apex. Among all *Cymbidium* spp., PDK/ORP-25 was the tallest with plant height 64.2cm and PDK/ORP-7 was smallest with plant height 9.6cm. The highest and lowest leaf length was measured in PDK/ORP-25(62.4cm) and PDK/ORP-7(15.2cm) respectively; the highest leaf width was observed in PDK/ORP-7 and 35 (2.3cm) while lowest was in PDK/ORP-24 (1.3cm). The accession number PDK/ORP-35 (3cm) measured highest internodal length, total number of leaves (30nos) and number of sprouts (18nos) whereas accession number PDK/ORP-7 (0.7 cm) showed lowest values for these traits. Internodal length was not measurable in PDK/ORP-24 and 25. Further the values obtained are classified as short, medium, long, broad, few and many as per the *Cymbidium* descriptor (Table 14) (Plate 7; Plate 8).

The absolute growth rate were recorded in percentage and highest absolute growth in plant height was observed in PDK/ORP-35 in March (27%). Absolute growth for leaf length was recorded as highest in PDK/ORP-7 in February (8%) and that of leaf width was highest in PDK/ORP-24 in January (30%). Internodal length recorded highest absolute growth in PDK/ORP-7 in February (33%) while the highest absolute growth for number of leaves was for PDK/ORP-25 in February (50%). Absolute growth in number of sprouts was observed highest in PDK/ORP-7 and PDK/ORP-24 in June (100%) (Fig.15-20).

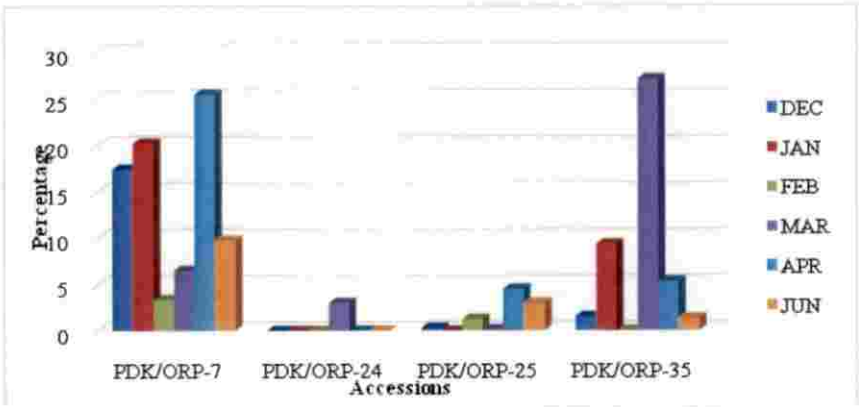


Figure 15. Absolute growth in plant height among the different *Cymbidium* species during 2015-16

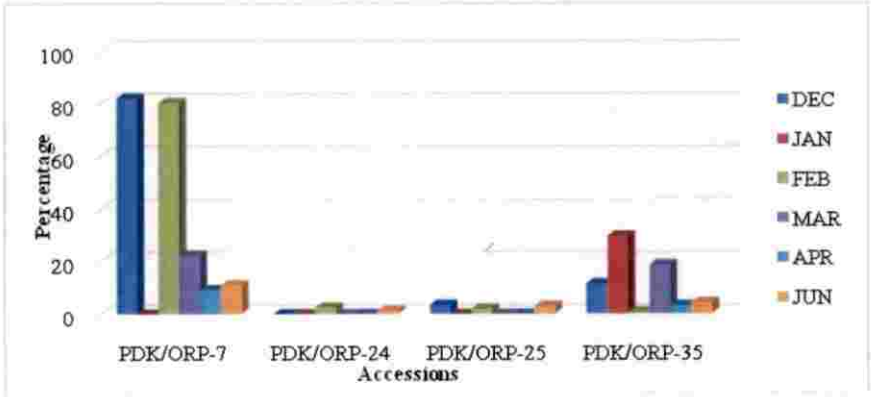


Figure 16. Absolute growth in leaf length among the different *Cymbidium* species during 2015-16

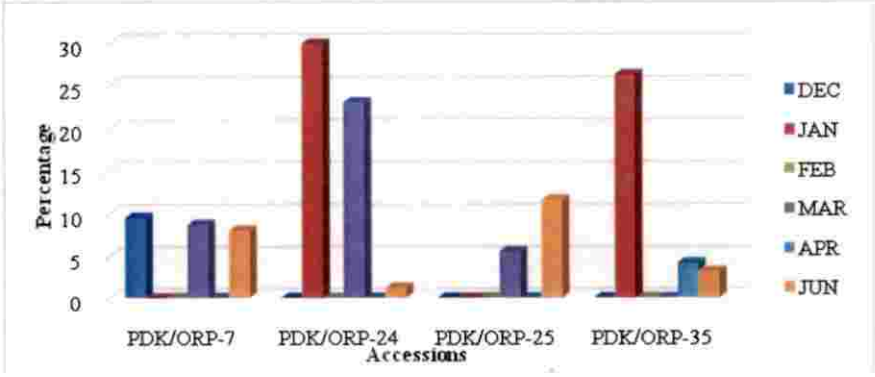


Figure 17. Absolute growth in leaf width among the different *Cymbidium* species during 2015-16

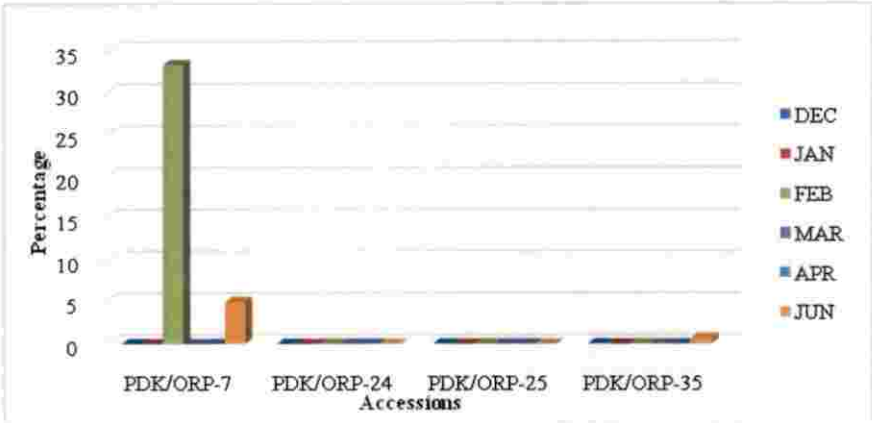


Figure 18. Absolute growth in intermodal length among the different *Cymbidium* species during 2015-16

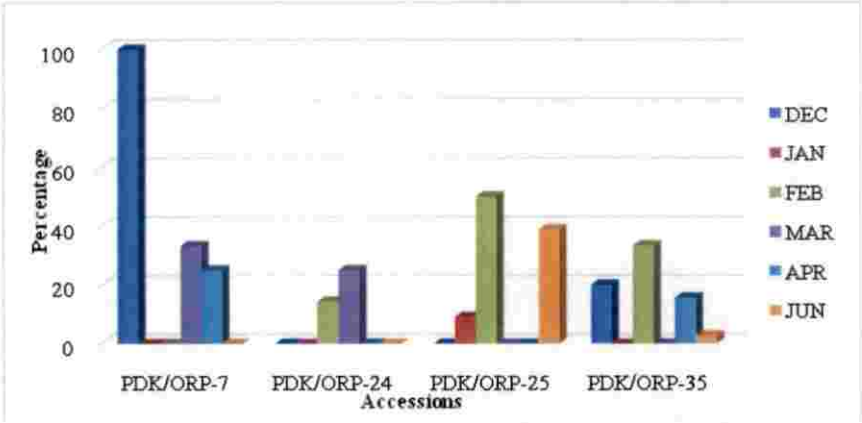


Figure 19. Absolute growth in number of leaves among the different *Cymbidium* species during 2015-16

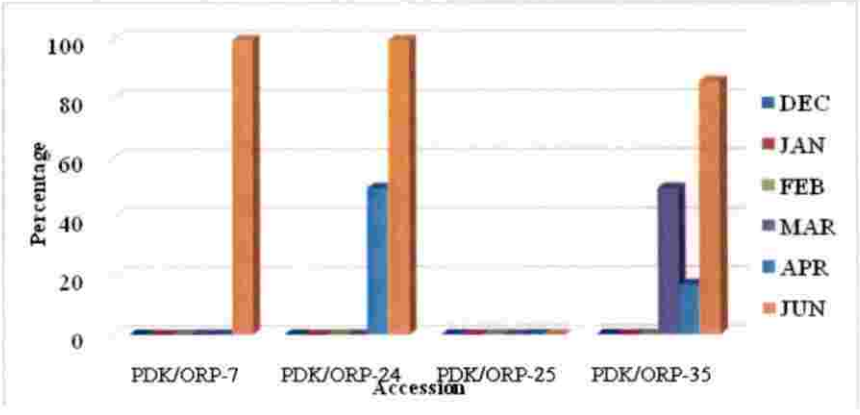


Figure 20. Absolute growth in number of sprouts among the different *Cymbidium* species during 2015-16

4.4.1.4 Bulbophyllum spp.

Bulbophyllum had 2 accessions which were similar in bulbous pseudo stem, elliptical leaf shape, and obtuse leaf apex. The two accessions varied with quantitative vegetative characters of which PDK/ORP-29 was the tallest with plant height 22.1 cm and the smallest was PDK/ORP-31 with plant height 9.5cm; the highest leaf length, number of leaves and was measured in PDK/ORP-29 (10.9cm) whereas lowest in PDK/ORP-31 (6.3cm). The highest and lowest leaf width was observed in PDK/ORP-31 (3.6cm) and PDK/ORP-29(3.5cm) respectively. The internodal length was 1.2 cm in PDK/ORP-29 whereas it was not measurable in accession PDK/ORP-31. Further the values obtained are classified as small, medium, large broad, and few as per the *Bulbophyllum* descriptor (Table 14)

The absolute growth rate was recorded in percentage and highest absolute growth in plant height was observed in PDK/ORP-29 in April (32%). Leaf length recorded highest absolute growth in PDK/ORP-29 in February (12%) and leaf width recorded highest absolute growth in PDK/ORP-31 in January (13%). There was no change in absolute growth in internodal length. Highest absolute growth for number of leaves (83%) and number of sprouts was observed in PDK/ORP-29 in June (300%) (Table 15).

4.4.1.5 Coelogyne spp.

Coelogyne has 2 accessions with vegetative characteristic varied in both accessions. Accession PDK/ORP-09 have fleshy stem with oblong shaped leaves, whereas accession PDK/ORP-28 with clave fleshy stem and linear oblong shaped leaves. Both the accessions had acute leaf apex.

Accession PDK/ORP-28 had the highest plant height (16.8cm), leaf length (13.3cm), and number of sprout (16nos) as compared to other. The accession PDK/ORP-09 observed with highest leaf width (2.8cm). Numbers of leaves (2

nos) were same in both. Further the values obtained are classified as small, medium, broad, and few as per the *Coelogyne* descriptor (Table 14).

Table 14. Quantitative traits (vegetative plant parts) and NRCO descriptor based classification of different accessions.

Sl. No.	Genotype	Accession code	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Internodal length (cm)	No of leaves	No of sprouts
1.	*Acampe praemorsa*	PDK/ORP-45	18.7 (Sm)	13.8 (M)	4.3 (B)	1.9 (Sm)	05 (F)	5
2.	*Aeridis maculosa*	PDK/ORP-30	16.1 (L)	08.9 (S)	3.4 (B)	0.0 -	04 (F)	5
3.	*Bulbophyllum elongatum*	PDK/ORP-29	22.1 (L)	10.9 (M)	3.5 (B)	1.2 (Sm)	05 (F)	6
4.	*Bulbophyllum fischeri*	PDK/ORP-31	09.5 (L)	06.3 (M)	3.6 (B)	0.0 -	01 (F)	20
5.	*Cattleya*	PDK/ORP-22	16.6 (M)	10.7 (M)	2.5 (M)	0.0 -	12 (F)	3
6.	*Coelogyne*	PDK/ORP-9	11.2 (Sm)	05.9 (S)	2.8 (B)	0.0 -	02 (F)	1
7.	*Coelogyne breviscapa*	PDK/ORP-28	16.8 (L)	13.3 (M)	1.5 (M)	0.0 -	02 (F)	16
8.	*Cymbidium*	PDK/ORP-7	09.6 (Sm)	15.2 (S)	2.3 (M)	0.7 (Sm)	07 (F)	2
9.	*Cymbidium*	PDK/ORP-24	33.4 (M)	40.5 (M)	1.3 (M)	0.0 -	08 (M)	7
10.	*Cymbidium*	PDK/ORP-25	64.2 (L)	62.4 (Lo)	1.8 (M)	0.0 -	14 (Ma)	0
11.	*Cymbidium*	PDK/ORP-35	31.2 (M)	28.6 (S)	2.3 (B)	3.0 (M)	30 (M)	18
12.	*Dendrobium*	PDK/ORP-8	13.3 (Sm)	10.2 (M)	2.3 (B)	1.9 (Sm)	08 (F)	1
13.	*Dendrobium* hybrid 1	PDK/ORP-11	25.6 (Sm)	13.3 (M)	4.1 (B)	4.0 (L)	09 (M)	0
14.	*Dendrobium* hybrid 2	PDK/ORP-13	51.4 (M)	09.0 (S)	4.8 (B)	3.0 (M)	11 (Ma)	1
15.	*Dendrobium* hybrid 3	PDK/ORP-15	49.1 (M)	14.4 (M)	6.5 (B)	3.5 (M)	05 (Ma)	1
16.	*Dendrobium* hybrid 4	PDK/ORP-16	46.6 (M)	06.6 (S)	5.4 (B)	3.0 (M)	11 (Ma)	2
17.	*Dendrobium* hybrid 5	PDK/ORP-17	49.2 (M)	15.0 (M)	4.8 (B)	3.3 (M)	15 (Ma)	4
18.	*Dendrobium* hybrid 6	PDK/ORP-18	15.1 (Sm)	17.0 (Lo)	5.4 (B)	0.0 -	15 (F)	2
19.	*Dendrobium*	PDK/ORP-20	43.9 (M)	08.4 (S)	3.9 (B)	3.7 (M)	03 (M)	5
20.	*Dendrobium aqueum*	PDK/ORP-34	20.7 (Sm)	11.0 (M)	1.3 (N)	2.2 (M)	06 (F)	1
21.	*Dendrobium* (T.C)	PDK/ORP-42	19.5 (Sm)	06.8 (S)	3.1 (B)	3.0 (M)	19 (F)	12

22.	*Flickingeria nodosa*	PDK/ORP-39	17.0 (M)	09.7 (S)	1.9 (M)	1.9 (Sm)	29 (F)	13
23.	Mini *Cattleya*	PDK/ORP-14	09.0 (Sm)	07.8 (S)	1.6 (N)	5.0 (L)	05 (F)	2
24.	*Oncidium tolumina*	PDK/ORP-12	14.2 (Sm)	13.0 (S)	0.6 (N)	0.0 -	10 (F)	0
25.	*Phalaenopsis* (T.C)	PDK/ORP-38	09.2 (L)	07.5 (S)	2.8 (M)	2.0 (Sm)	03 (F)	0
26.	*Phalenopsis hybrid*	PDK/ORP-10	29.8 (L)	25.0 (Lo)	8.3 (B)	1.4 (Sm)	04 (M)	0
27.	*Pholidota imbricata*	PDK/ORP-32	21.5 (M)	16.6 (M)	3.3 (B)	2.5 (M)	03 (M)	7
28.	*Rhynchostylis retusa*	PDK/ORP-33	61.0 (L)	14.6 (M)	3.3 (B)	1.6 (Sm)	10 (Ma)	2
29.	*Vanda testacea*	PDK/ORP-1	48.4 (M)	15.8 (M)	2.6 (B)	3.8 (Lo)	13 (M)	5
30.	*Vanda*	PDK/ORP-2	62.8 (L)	14.5 (M)	2.6 (B)	2.7 (Lo)	23 (Ma)	6
31.	*Vanda*	PDK/ORP-3	66.4 (L)	37.9 (Lo)	2.5 (B)	2.7 (Lo)	30 (Ma)	4
32.	*Vanda*	PDK/ORP-4	95.1 (VL)	13.7 (M)	3.0 (B)	3.0 (Lo)	32 (Ma)	4
33.	*Vanda*	PDK/ORP-5	126 (VL)	13.8 (M)	2.0 (B)	2.9 (Lo)	41 (Ma)	4
34.	*Vanda*	PDK/ORP-6	5.9 (VSm)	05.0 (S)	0.9 (M)	0.0 -	05 (F)	1
35.	*Vanda*	PDK/ORP-19	15.3 (Sm)	28.3 (Lo)	3.4 (B)	4.0 (Lo)	09 (F)	0
36.	*Vanda*	PDK/ORP-21	46.7 (M)	13.7 (M)	2.2 (B)	3.5 (Lo)	20 (Ma)	2
37.	*Vanda*	PDK/ORP-23	26.0 (Sm)	10.5 (M)	1.4 (B)	0.5 (S)	06 (F)	4
38.	*Vanda*	PDK/ORP-36	14.0 (Sm)	14.0 (M)	1.3 (B)	1.0 (M)	05 (F)	4
39.	*Vanda*	PDK/ORP-43	28.7 (Sm)	17.8 (M)	1.8 (B)	2.8 (Lo)	12 (M)	6
40.	*Vanda*	PDK/ORP-44	54.2 (M)	18.2 (M)	2.7 (B)	3.3 (Lo)	11 (M)	0
41.	*Vanda*	PDK/ORP-46	26.4 (Sm)	15.1 (M)	2.1 (B)	4.0 (Lo)	04 (F)	0

(B: Broad; F: Few; L: Large, Lo: Long; M: Medium; Ma: Many; N: Narrow; S: Short; Sm: Small; VL: Very Large; VSm: Very Small)

The absolute growth rate was recorded in percentage and highest absolute growth for plant height was observed in PDK/ORP-9 in April (9%). Leaf length recorded highest absolute growth in PDK/ORP-9 in February (25%) while highest absolute growth for leaf width was observed in PDK/ORP-9 in June (32%). There was no change in absolute growth in internodal length. Highest absolute growth for number of leaves was observed in PDK/ORP-9 in February (50%) and the highest absolute growth for number of sprout was recorded in PDK/ORP-9 in June (100%) (Table 15).

4.4.1.6 Phalaenopsis spp.

Phalaenopsis had 2 accessions, one tissue culture plant obtained through pod culture and one hybrid; both having fleshy stem, oblong shaped leaf and acute leaf apex. Accession PDK/ORP-10 was highest compared to the other accession in plant height (29.8cm), leaf length (25.0cm), and leaf width(8.3cm), total number of leaves (4nos) and internodal length (2.0cm) was observed highest in PDK/ORP-38, whereas there were no sprouts observed in both the accessions). Further the value obtained are classified as small, medium, large, long, broad, few and medium as per the *Phalaenopsis* descriptor (Table 14) (Plate 7).

The absolute growth rate was recorded in percentage and highest absolute growth for height was observed in PDK/ORP-38 in December (28%). Leaf length recorded highest absolute growth in PDK/ORP-10 in June (1%) while that for leaf width was in PDK/ORP-38 in March (28%). The absolute growth in internodal length was highest in PDK/ORP-10 March (29%). Highest absolute growth for number of leaves was observed in PDK/ORP-38 in January (200%), and the absolute growth for number of sprout remained unchanged as (0%) in both the accessions of *Phalenopsis* (Table 15).

The rest of 8 genera comprise of 1 accession each, namely: *Oncidium tolumina*, Mini *Cattleya*, *Cattleya*, *Aeridis maculosa*, *Pholidota imbricata*, *Rhynchostylis retusa*, *Flickingeria nodosa*, and *Acampe praemorsa* (PDK/ORP-12, PDK/ORP-14, PDK/ORP-22, PDK/ORP-30, PDK/ORP-32, PDK/ORP-33,

PDK/ORP-39, and PDK/ORP-45.) Further the value obtained are classified as small, medium, large, short, long, narrow, broad, few, and many as per their descriptors (Table 14). (Plate 7, Plate 8).

4.4.1.7 Oncidium tolumina (PDK/ORP-12)

The absolute growth rate (%) for plant height was highest in December (49%), for leaf length (6%) and leaf width (3%) in June, while for number of leaves it was in January (67%). There was no absolute growth in internodal length and number of sprout (Table 15).

4.4.1.8 Mini *Cattleya* (PDK/ORP-14)

The absolute growth rate (%) for plant height was highest in March (20%), for leaf length (68%) and leaf width (9%) in June, while for number of leaves it was in March (20%). For internodal length it was highest in December (6%) and for number of sprout, in March (20%) (Table 15).

4.4.1.9 Cattleya (PDK/ORP-22)

The absolute growth rate (%) for plant height was highest in April (12%), for leaf length in March (5%) while for leaf width (2%) and number of leaves (33%) it was in June. For internodal length there was no change while for number of sprout it was in March (100%) (Table 15).

Table 15: Absolute growth percentage for 11 accessions during December 2015 to June16.

Accession	Absolute growth (%)	DEC	JAN	FEB	MAR	APR	JUN
Acampe praemorsa (PDK/ORP-45)	Plant height	0	11	15	17	10	11
	Leaf length	2	5	16	0	0	1
	Leaf width	15	89	88	5	0	6
	Internodal length	0	0	6	5	0	3
	No. of leaves	0	0	0	0	20	17
	Sprouts	0	0	0	100	0	100
Aerides maculosa (PDK/ORP-30)	Plant height	7	15	0	13	0	0
	Leaf length	0	1	1	0	0	10
	Leaf width	10	6	0	0	0	37
	Internodal length	0	0	0	0	0	0
	No. of leaves	0	300	0	25	0	40
	Sprouts	0	0	0	0	0	50
B. elongatum (PDK/ORP-29)	Plant height	0	9	1	11	32	2
	Leaf length	0	0	12	3	9	30
	Leaf width	0	0	0	0	0	9
	Internodal length	0	0	0	0	0	0
	No. of leaves	0	20	0	0	0	83
	Sprouts	0	0	0	0	0	300
B. fischeri (PDK/ORP-31)	Plant height	9	12	8	7	22	4
	Leaf length	0	0	8	0	0	5
	Leaf width	0	13	6	0	0	0
	Internodal length	0	0	0	0	0	0
	No. of leaves	0	0	0	0	0	0
	Sprouts	0	0	0	0	0	0
Cattleya (PDK/ORP-22)	Plant height	7	3	5	4	12	3
	Leaf length	0	0	0	5	0	4
	Leaf width	0	0	0	0	0	2
	Internodal length	0	0	0	0	0	0
	No. of leaves	0	0	0	0	0	33
	Sprouts	0	0	0	0	0	100
Coelogyne (PDK/ORP-9)	Plant height	0	6	8	0	9	3
	Leaf length	0	8	25	14	0	1
	Leaf width	22	0	0	11	0	32
	Internodal length	0	0	0	0	0	0
	No. of leaves	0	0	50	33	0	0
	Sprouts	0	0	0	0	0	100
Coelogyne breviscapa (PDK/ORP-28)	Plant height	0	0	3	0	1	1
	Leaf length	0	0	2	0	0	0
	Leaf width	0	0	0	0	0	13
	Internodal length	0	0	0	0	0	0
	No. of leaves	0	0	0	0	0	0
	Sprouts	0	0	0	25	0	60

Flickingeria nodosa (PDK/ORP-39)	Plant height	0	11	20	0	11	7
	Leaf length	2	10	1	0	2	4
	Leaf width	0	12	0	5	10	0
	Internodal length	0	25	0	0	0	1
	No. of leaves	0	0	0	0	0	0
	Sprouts	0	0	0	60	50	0
Mini *Cattleya* (PDK/ORP-14)	Plant height	0	0	0	20	0	0
	Leaf length	21	0	0	0	0	68
	Leaf width	0	0	0	0	0	9
	Internodal length	6	0	0	0	0	1
	No. of leaves	0	0	0	20	0	0
	Sprouts	0	0	0	20	0	0
Oncidium tolumina (PDK/ORP-12)	Plant height	49	28	20	13	23	8
	Leaf length	0	0	0	0	0	6
	Leaf width	0	0	0	0	0	3
	Internodal length	0	0	0	0	0	0
	No. of leaves	0	67	0	0	30	0
	Sprouts	0	0	0	0	0	0
Phalaenopsis (T.C) (PDK/ORP-38)	Plant height	28	0	0	0	0	0
	Leaf length	0	0	0	0	0	0
	Leaf width	4	16	24	28	15	22
	Internodal length	0	0	0	0	0	0
	No. of leaves	0	200	0	33	0	0
	Sprouts	0	0	0	0	0	0
Phalenopsis hybrid (PDK/ORP-10)	Plant height	3	7	4	6	0	0
	Leaf length	0	0	0	0	0	1
	Leaf width	0	1	0	4	0	2
	Internodal length	0	20	17	29	0	4
	No. of leaves	0	0	0	0	40	0
	Sprouts	0	0	0	0	0	0
Pholidota imbricata (PDK/ORP-32)	Plant height	0	6	16	11	0	0
	Leaf length	4	3	3	28	0	13
	Leaf width	4	21	0	0	0	4
	Internodal length	0	0	0	0	0	3
	No. of leaves	0	200	0	0	67	0
	Sprouts	0	0	0	0	0	50
Rhynchostylis retusa (PDK/ORP-33)	Plant height	0	0	0	0	0	0
	Leaf length	0	0	7	1	0	4
	Leaf width	0	0	3	0	0	5
	Internodal length	7	0	0	0	0	1
	No. of leaves	0	0	11	0	0	0
	Sprouts	0	0	0	0	0	0

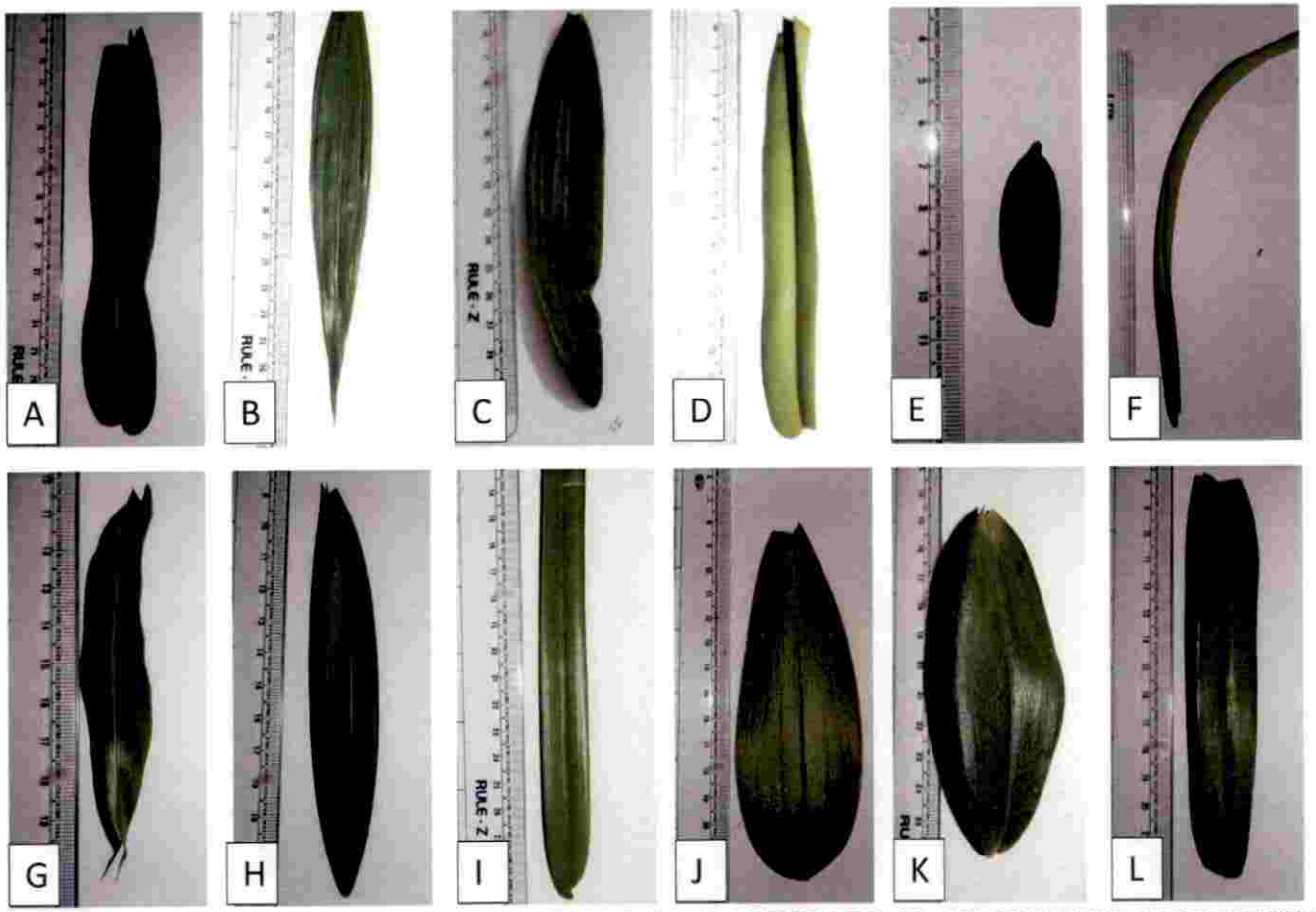


Plate 7: Different shapes of leaves in accession (A) PDK/ORP-30, (B) PDK/ORP-07, (C) PDK/ORP-32,(D) PDK/ORP-44, (E) PDK/ORP-14, (F) PDK/ORP-03, (G) PDK/ORP-34, (H) PDK/ORP-39, (I) PDK/ORP-35, (J) PDK/ORP-38, (K) PDK/ORP-15, (L) PDK/ORP-01.

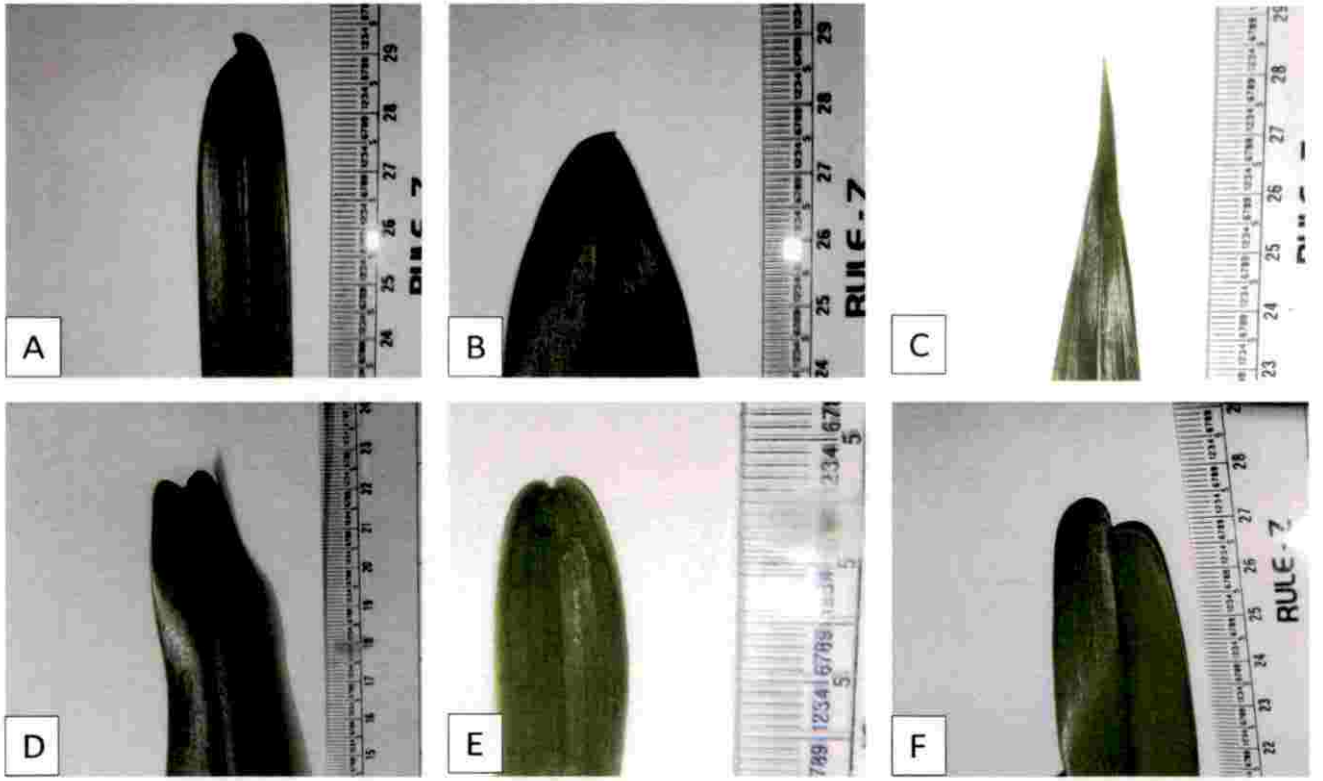


Plate 8: variation in leaf apex of different accessions (A) PDK/ORP-35, (B) PDK/ORP-18, (C) PDK/ORP-07, (D) PDK/ORP-03, (E) PDK/ORP-05, (F) PDK/ORP-30.

4.4.1.10 Aeridis maculosa (PDK/ORP-30)

The absolute growth rate for plant height (15%) and number of leaves (300%) was highest in January (15%) while that for leaf length (10%) and leaf width (37%), in June. For internodal length there was no change while for number of sprout it was in June (50%) (Table 15).

4.4.1.11 Pholidota imbricate (PDK/ORP-32)

The absolute growth rate (%) for plant height was highest in February (16%), for leaf length in March (28%), for leaf width (21%) and number of leaves (200%) in January. For internodal length there was no change while for number of sprout it was in June (50%) (Table 15).

4.4.1.12 Rhynchostylis retusa (PDK/ORP-33)

The absolute growth rate (%) for leaf width was highest during June (5%) and number of leaves in January (11%). For plant height, internodal length and number of sprouts there was no change (Table 15).

4.4.1.13 Flickingeria nodosa (PDK/ORP-39)

The absolute growth rate (%) for plant height was highest in February (20%), for leaf length (10%), leaf width (12%) and internodal length (25%) in January. For internodal length and number of leaves there was no change while for number of sprout it was in March (60%) (Table 15).

4.4.1.14 Acampe praemorsa (PDK/ORP-45)

The absolute growth rate (%) for plant height (17%) and number of sprout (100%) was highest in March, for leaf length (16%) and internodal length (6%) in February, for leaf width in January (89%) and number of leaves (20%) in April (Table 15).

4.4.2 Reproductive characters

The observations on reproductive characters were recorded at flowering phase. Out of the 14 genera, only two reached the flowering stage *viz*., *Dendrobium* (5 accessions) and *Acampe* (one accession viz., *A. praemorsa*, (PDK/ORP-45). In *Dendrobium* accessions, four were hybrids namely *Dendrobium* hybrid 1, hybrid 2, hybrid 3 and hybrid 4- PDK/ORP-11,13,15 and 16 respectively and one species collected from Brahmagiri (PDK/ORP-20).

All the 6 accessions had two petals, one lip, one dorsal sepal, and two lateral sepals. Among the *Dendrobium* accessions, Accession PDK/ORP-20 had highest petal length, dorsal sepal length, as well as length and width of lip, while hybrid 4 (PDK/ORP-16) had the highest petal width, dorsal sepal width and length and width of lateral sepal (Table 16). (PDK/ORP-15) had the lowest petal length, dorsal sepal length, length, and width of lateral sepal and length of the lip (PDK/ORP-13) was with lowest petal width while PDK/ORP-20 was with lowest dorsal sepal length and PDK/ORP-11 with lowest lip width In *A. praemorsa* (PDK/ORP-45), the flowers were very small petals, sepals, and lip compared to those of *Dendrobium* (Table 16) (Plate 9).

The petal shape varied in the accessions present with 3 obovate (PDK/ORP-11, PDK/ORP-15, PDK/ORP-45), 2 orbicular (PDK/ORP-13, PDK/ORP-16) and 1 elliptical (PDK/ORP-20) (Table 17). The petal curvature was deflexed in all the 6 accessions. The dorsal sepal shape of the 5 accessions was elliptical while in PDK/ORP-20 it was oblong. The lateral sepal shape was triangular, lip shape orbicular and lip margin entire for all the 5 accessions of *Dendrobium* while for *A. praemorsa* lateral sepal shape and lip shape was obovate and lip margin was undulated. All the 4 hybrids of *Dendrobium* have apex of petal as retuse, while accession from Brahmagiri (PDK/ORP-20) has acute apex of petal. However, all the 5 accession of *Dendrobium* have apex of dorsal and lateral sepal as acute whereas for *Acampe praemorsa* (PDK/ORP-45), the petal and sepal apex were obtuse.

The apex of the lip was retuse for 4 accessions of *Dendrobium* while for Brahmagiri accession of *Dendrobium* (PDK/ORP-20) and *Acampe praemorsa* (PDK/ORP-45) lip apex was obtuse. Texture of the lip surface was glabrous for the 5 accessions of *Dendrobium* and pubescent for *Acampe praemorsa.* The flower duration varied for each accession. The longest flower duration was observed in the *Dendrobium* accession PDK/ORP-20 with 26 days and lowest flower duration was observed in the accession PDK/ORP-13 with 8 days.

4.4.3 Palynological characters

The Palynological characters were studied for all the 6 accessions. All the *Dendrobium* accessions had 4 pollinia while *Acampe praemorsa* had 2 pollinia (Table 18). Pollinia shape varied with 2 lentiform (PDK/ORP-11and PDK/ORP-13), 3 coniform (PDK/ORP-15, PDK/ORP-16, and PDK/ORP-20), and one hemispherical (PDK/ORP-45). The colour of the pollinia was similar in all 5 accessions *of Dendrobium* (light yellow) whereas in *Acampe praemorsa* (PDK/ORP-45), it was yellow. All the 6 accessions had similar pollen grain shape (round, oval) and pollen viability as 100% (Plate 10; Plate 11; Plate 12).

Table 16. Floral morphology (quantitative traits) of six accessions of orchids in orchidarium.

Sl. No.	Genotype	Accession code	Petals		Dorsal sepals		Lateral sepals		Lip	
			Length (mm)	Width (mm)	Length (mm)	Width (mm)	Length (mm)	Width (mm)	Length (mm)	Width (mm)
1	*Dendrobium* hybrid 1	PDK/ORP-11	32.7	21.4	28.1	14.4	27.8	15.5	30.7	15.8
2	*Dendrobium* hybrid 2	PDK/ORP-13	34.6	17.8	29.1	14.2	32.3	16.1	31.2	16.5
3	*Dendrobium* hybrid 3	PDK/ORP-15	27.3	18.5	23.3	14.1	24.9	14.4	26.2	17.1
4	*Dendrobium* hybrid 4	PDK/ORP-16	38.9	44.1	34.8	18.1	36.4	19.9	32.1	18.8
5	*Dendrobium* sp.	PDK/ORP-20	45.7	35.5	35.6	12.3	36.1	15.4	34.2	21.8
6	*Acampe praemorsa*	PDK/ORP-45	9.2	2.2	7.5	4.1	8.2	4.1	8.3	4.5

Table 17. Floral morphology (descriptive traits) of six accessions of orchids in orchidarium.

Sl. No.	Genotype	Accession code	Petal shape	Petal curvature	Dorsal sepal shape	Lateral sepal shape	Lip shape	Lip margin
1	*Dendrobium* hybrid 1	PDK/ORP-11	Obovate	Deflexed	Elliptic	Triangular	Orbicular	Entire
2	*Dendrobium* hybrid 2	PDK/ORP-13	Orbicular	Deflexed	Elliptic	Triangular	Orbicular	Entire
3	*Dendrobium* hybrid 3	PDK/ORP-15	Obovate	Deflexed	Elliptic	Triangular	Orbicular	Entire
4	*Dendrobium* hybrid 4	PDK/ORP-16	Orbicular	Deflexed	Elliptic	Triangular	Orbicular	Entire
5	*Dendrobium* sp.	PDK/ORP-20	Elliptic	Deflexed	Oblong	Triangular	Orbicular	Entire
6	*Acampe praemorsa*	PDK/ORP-45	Obovate	Deflexed	Elliptic	Obovate	obovate	Undulated

Table 17 (continued). Floral morphology (descriptive traits) of six accessions of orchids in orchidarium.

Sl. No.	Genotype	Accession code	Apex of Petal	Apex of Dorsal sepal	Apex of Lateral sepal	Apex of Lip	Lip surface texture	Flower duration (No. of days)
1	*Dendrobium* hybrid 1	PDK/ORP-11	Retuse	Acute	Acute	Retuse	Glabrous	13
2	*Dendrobium* hybrid 2	PDK/ORP-13	Retuse	Acute	Acute	Retuse	Glabrous	8
3	*Dendrobium* hybrid 3	PDK/ORP-15	Retuse	Acute	Acute	Retuse	Glabrous	12
4	*Dendrobium* hybrid 4	PDK/ORP-16	Retuse	Acute	Acute	Retuse	Glabrous	17
5	*Dendrobium* sp.	PDK/ORP-20	Acute	Acute	Acute	Obtuse	Glabrous	26
6	*Acampe praemorsa*	PDK/ORP-45	Obtuse	Obtuse	Obtuse	Obtuse	Pubescent	10

Table 18: Palynology of six accessions of orchids in orchidarium.

Sl No.	Genotype	Accession code	Number of pollinia	Pollinia shape	Pollinia colour	Pollen grain shape	Pollen viability (%)
1	*Dendrobium* hybrid 1	PDK/ORP-11	4	lentiform	light yellow	Round, oval	100
2	*Dendrobium* hybrid 2	PDK/ORP-13	4	lentiform	light yellow	Round, oval	100
3	*Dendrobium* hybrid 3	PDK/ORP-15	4	coniform	light yellow	Round, oval	100
4	*Dendrobium* hybrid 4	PDK/ORP-16	4	coniform	light yellow	Round, oval	100
5	*Dendrobium* sp.	PDK/ORP-20	4	coniform	light yellow	Round, oval	100
6	*Acampe praemorsa*	PDK/ORP-45	2	hemispherical	yellow	Round, oval	100

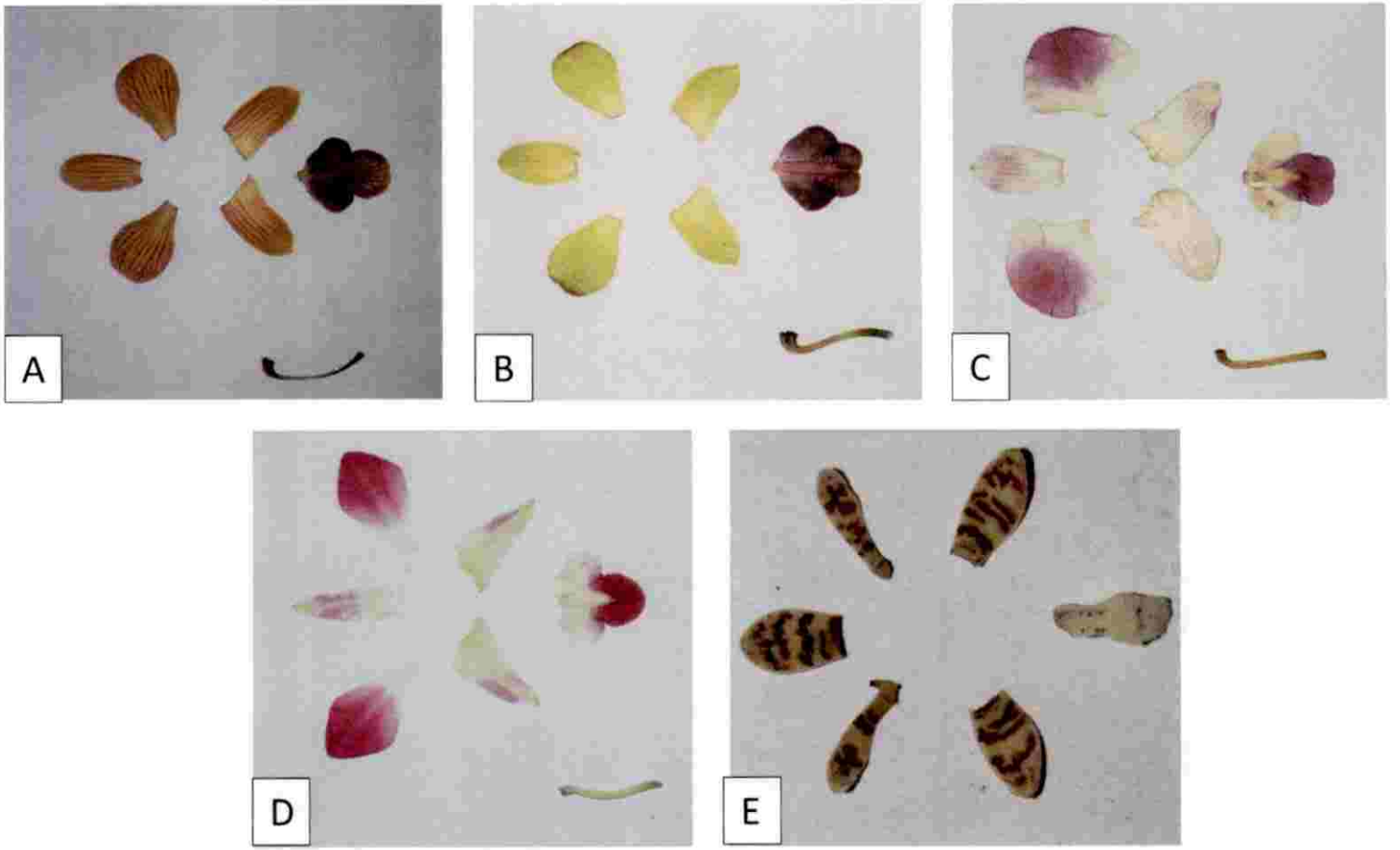


Plate 9: Arrangement of petals, dorsal sepal, lateral sepal and lip for accession (A) PDK/ORP-11, (B) PDK/ORP-15, (C) PDK/ORP-16, (D) PDK/ORP-20, (E) PDK/ORP-45.

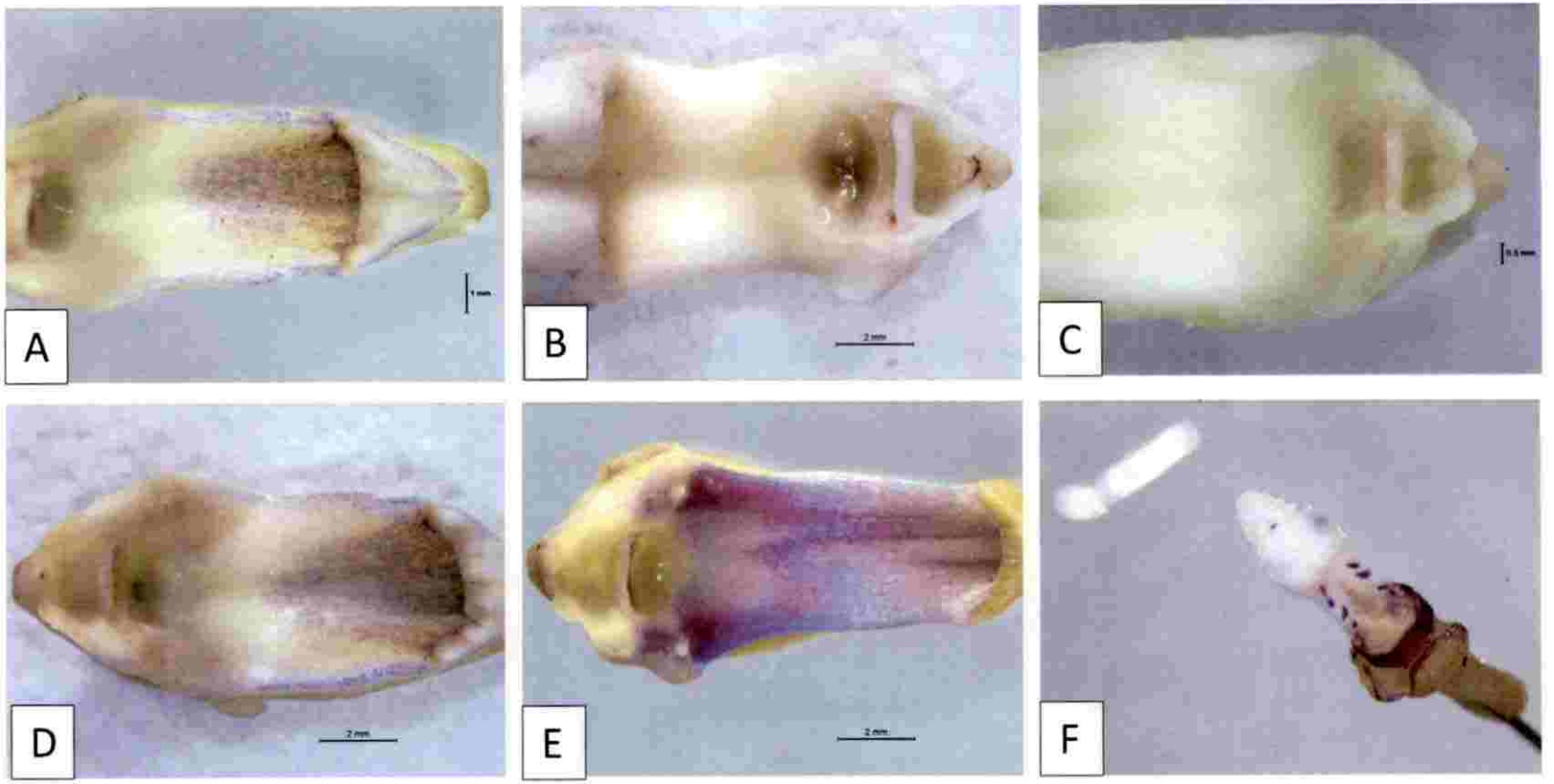


Plate 10: Different shape and colour of column (A) PDK/ORP-11, (B) PDK/ORP-13, (C) PDK/ORP-15, (D) PDK/ORP-16, (E) PDK/ORP-20, (F) PDK/ORP-45.

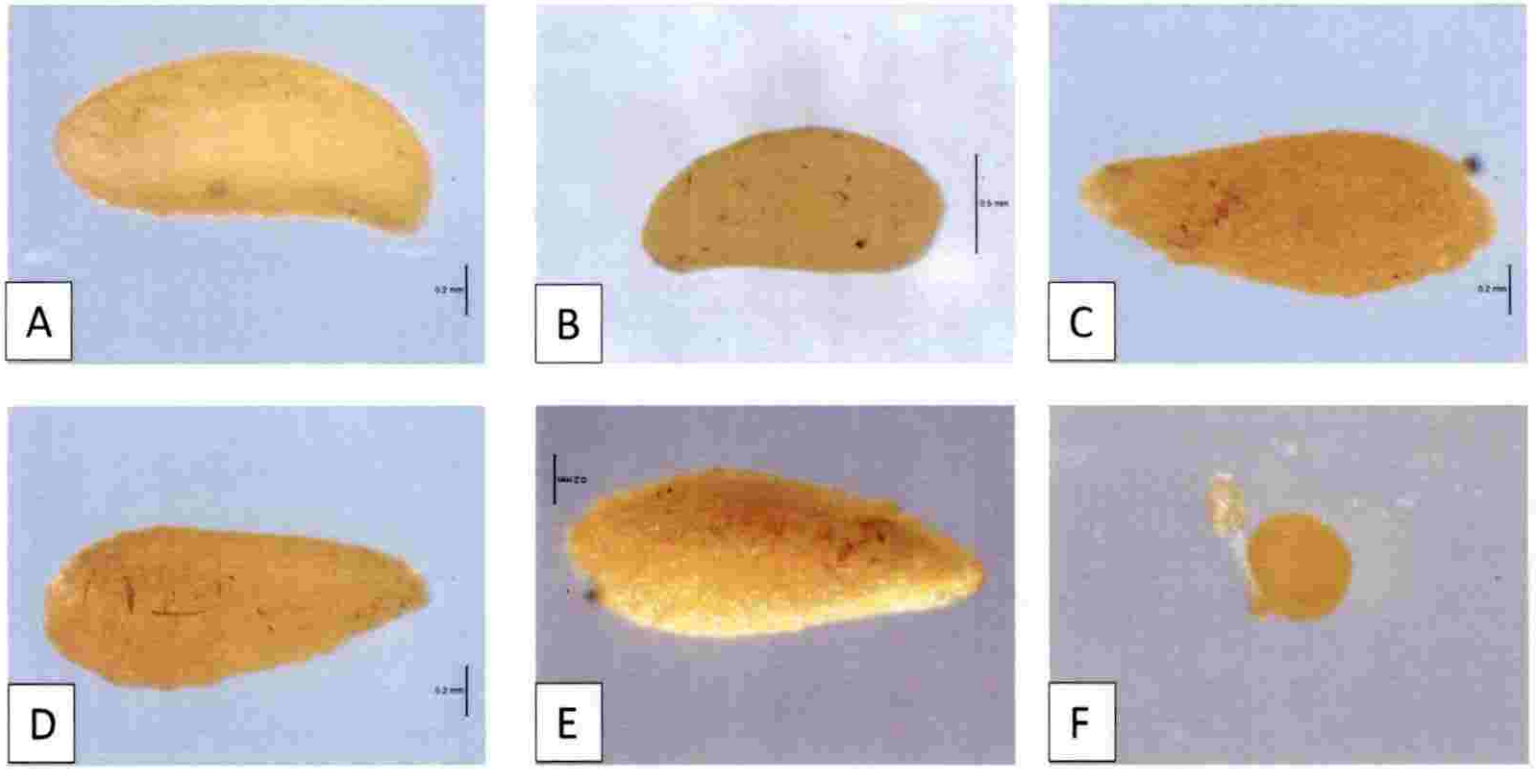


Plate 11: Shape of pollinia (A) PDK/ORP-11, (B) PDK/ORP-13, (C) PDK/ORP-15, (D) PDK/ORP-16, (E) PDK/ORP-20, (F) PDK/ORP-45.

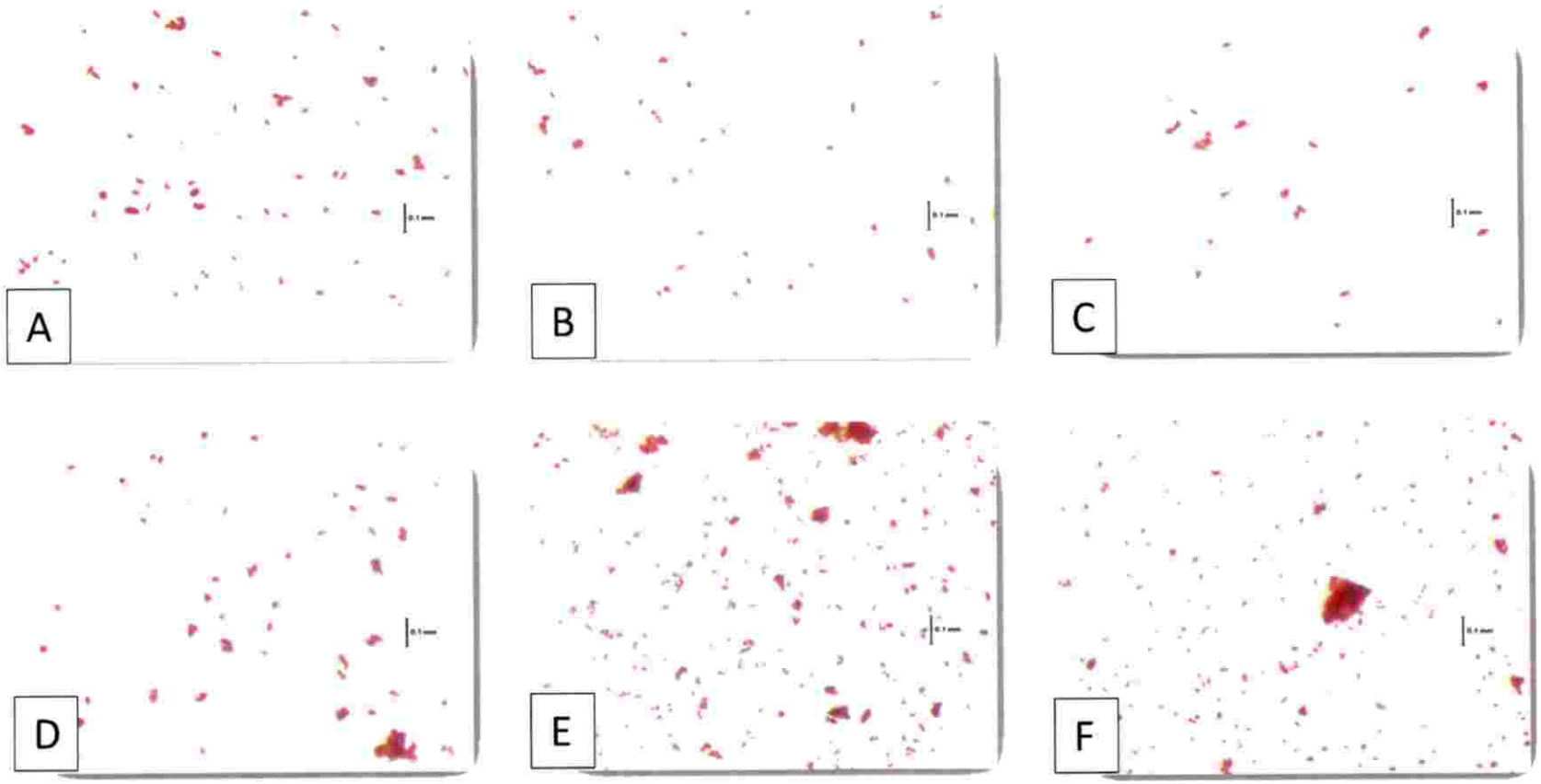


Plate 12: Viable pollen grain (A) PDK/ORP-11, (B) PDK/ORP-13, (C) PDK/ORP-15, (D) PDK/ORP-16, (E) PDK/ORP-20, (F) PDK/ORP-45.

4.5 MOLECULAR CHARACTERIZATION USING RAPD

Genomic DNA was isolated from 40 accessions out of 46, the quality and quantity of isolated DNA samples were estimated, and selected samples were subjected to RAPD analysis.

Molecular characterization was done as two experiments. First experiment was for detecting inter-generic polymorphism. For this, 17 accessions belonging to 14 genera were selected so that at least one representative of each genus is included for amplification using 10 selected decamer primers (Table 2).

The second experiment was for detecting intra-generic polymorphism and duplicates, if any, in the collection. For this, 9 accessions each from genera, *Vanda* and *Dendrobium* were separately amplified using selected 5 primers from the 10primers used in inter-generic comparison.

4.5.1 DNA isolation

High molecular weight genomic DNA was isolated from 40 samples out of 46 accessions of orchids (Table19), as in the remaining six accessions *viz*., Accession No. PDK/ORP-6, 40 and 41 (*Vanda* spp.); PDK/ORP-26 and 27 (*Cymbidium* spp.); PDK/ORP-37 (*Rhynchostylis retusa*), the vegetative part available for DNA extraction was not sufficient.

4.5.2 Quantification of DNA samples

The quality and quantity of the DNA was assessed using gel electrophoresis and biophotometer (Plate 3, Table 19).

The extracted DNA was pure but the presence of mucilage substances slightly reduced the quality as indicated by the absorbance ratio. However, the electrophoresis revealed a single high molecular weight band without any degradation (Plate 3).

Table 19: Quality and quantity of DNA isolated from orchid accessions.

Sl. No.	Name of species	Accession code	Absorbance ratio ($A_{260/280}$)	Concentration µg/µl	Selection for RAPD analysis
1.	*Acampe praemorsa*	PDK/ORP-45	1.42	104.3	selected
2.	*Aeridis maculosa*	PDK/ORP-30	1.24	19.6	Selected
3.	*B. elongatum*	PDK/ORP-29	1.29	23.0	Selected
4.	*B. fischeri*	PDK/ORP-31	1.30	40.3	Selected
5.	*Cattleya*	PDK/ORP-22	1.31	36.0	Selected
6.	*Coelogyne*	PDK/ORP-9	1.27	22.2	
7.	*Coelogyne breviscapa*	PDK/ORP-28	1.52	76.8	Selected
8.	*Cymbidium*	PDK/ORP-7	1.33	29.7	Selected
9.	*Cymbidium*	PDK/ORP-24	1.67	118.4	Selected
10.	*Cymbidium*	PDK/ORP-25	1.61	145.8	
11.	*Cymbidium*	PDK/ORP-26	-	-	
12.	*Cymbidium*	PDK/ORP-27	-	-	
13.	*Cymbidium*	PDK/ORP-35	1.69	97.7	Selected
14.	*Dendrobium*	PDK/ORP-8	1.73	138.2	
15.	*Dendrobium*	PDK/ORP-20	1.27	50.2	
16.	*Dendrobium (T.C)*	PDK/ORP-42	1.33	29.6	
17.	*Dendrobium aqueum*	PDK/ORP-34	1.73	93.9	
18.	*Dendrobium* hybrid 1	PDK/ORP-11	1.06	5.5	
19.	*Dendrobium* hybrid 2	PDK/ORP-13	1.68	27.7	
20.	*Dendrobium* hybrid 3	PDK/ORP-15	1.25	24.0	
21.	*Dendrobium* hybrid 4	PDK/ORP-16	1.29	33.1	
22.	*Dendrobium* hybrid 5	PDK/ORP-17	1.34	32.7	
23.	*Dendrobium* hybrid 6	PDK/ORP-18	1.27	50.8	Selected
24.	*Flickingeria nodosa*	PDK/ORP-39	1.62	59.6	Selected
25.	Mini *Cattleya*	PDK/ORP-14	1.57	79.4	Selected
26.	*Oncidium tolumina*	PDK/ORP-12	1.13	86.7	Selected
27.	*Phalaenopsis (T.C)*	PDK/ORP-38	1.44	28.1	
28.	*Phalenopsis* hybrid	PDK/ORP-10	1.71	28.0	Selected
29.	*Pholidota imbricata*	PDK/ORP-32	1.64	20.7	Selected
30.	*Rhynchostylis retusa*	PDK/ORP-33	1.61	57.0	Selected
31.	*Rhynchostylis retusa*	PDK/ORP-37	-	-	
32.	*Vanda testacea*	PDK/ORP-1	1.73	140.2	Selected
33.	*Vanda*	PDK/ORP-3	1.17	58.9	
34.	*Vanda*	PDK/ORP-4	1.60	15.7	

35.	*Vanda*	PDK/ORP-5	1.48	21.3	
36.	*Vanda*	PDK/ORP-6	-	-	
37.	*Vanda*	PDK/ORP-19	1.53	36.6	
38.	*Vanda*	PDK/ORP-21	1.72	85.9	
39.	*Vanda*	PDK/ORP-23	1.87	39.1	
40.	*Vanda*	PDK/ORP-36	1.50	129.7	
41.	*Vanda*	PDK/ORP-40	-	-	
42.	*Vanda*	PDK/ORP-41	-	-	
43.	*Vanda*	PDK/ORP-43	1.31	29.4	
44.	*Vanda*	PDK/ORP-44	1.28	39.3	
45.	*Vanda*	PDK/ORP-46	1.42	81.8	
46.	*Vanda*	PDK/ORP-2	1.68	27.7	

The quantified DNA samples showed differences in the yield and in the $A_{260/280}$ ratio values. Samples with good quality and quantity and representing the fourteen genera were picked for further molecular analysis (Table 19). However, two species from genus *Bulbophyllum* and three genotypes from *Cymbidium* were also included in order to check the polymorphism within these genera.

4.5.3 Primer screening

In the preliminary step of the experiment, primer screening was conducted with 30 RAPD primers with two distinct orchid accessions *viz.*, *Phalenopsis* hybrid (PDK/ORP-10), and *Cymbidium* sp. (PDK/ORP-24). Out of the 30 primers used, 10 of the primers (OPA 5, OPA 11, OPA 16, OPA 18, OPA 20, OPAW 5, OPAW 12, OPBA 3, OPE12, and OPM15) responded with a good banding pattern with high polymorphic bands and these selected primers were used to perform RAPD profiling of the selected 17 accessions of orchids. PCR amplifications were resolved by agarose gel (1.5%) electrophoresis and the documentation was done using Bio-Rad gel documentation unit.

The screening results helped to pick the most efficient primers giving the maximum amplification in the experiment based on the number of amplicons and

banding pattern, a total of 10 RAPD primers were selected for further analysis (Table 2).

4.5.4 RAPD profiling for detecting intergeneric polymorphism

Further, the 10 efficient RAPD primers were used to study the diversity of the selected orchids accessions. The amplification pattern includes polymorphic and monomorphic bands. The banding pattern yielded by the primers was unique for each sample. These 10 primers produced a total of 399 scorable DNA fragments of high polymorphism. The results obtained from the 10 primers are detailed below (Table 20).

OPA 05 *(AGGGGTCTTG)*

The primer OPA 05 gave a good amplification pattern in all the 17 accessions tested. There were a total of 37 polymorphic and without any monomorphic bands. Some notable specific bands were observed in some accessions, which are absent in all other accessions (Plate 13).

OPA 11 *(CAATCGCCGT)*

The primer OPA 11 gave a good amplification in the all the 17 accession tested. There were a total of 43 polymorphic and without any monomorphic bands. Some notable specific bands were present in the accession which are absent in all the other accessions (Plate 14).

OPA 16 *(AGCCAGCGAA)*

The primer OPA 16 gave a good amplification in the all the 17 accession tested. There were a total of 39 polymorphic and without any monomorphic bands. This primer failed to produce any diagnostic species specific bands. However there is some common band in some of the species (Plate 15).

OPA 18 *(AGGTGACCGT)*

The primer OPA 18 gave a good amplification in the all the 17 accession tested. There were a total of 41 polymorphic and without any monomorphic bands. The primer was efficient and contributed 6 species specific bands. Some notable specific bands were observed in some accessions, which are absent in all other accessions (Plate 16).

OPA 20 *(GTTGCGATCC)*

The primer OPA 20 gave a good amplification in the all the 17 accession tested. There were a total of 35 polymorphic and without any monomorphic bands. Some notable specific bands were observed in some accessions, which are absent in all other accessions (Plate 17).

OPAW 05 *(CTGCTTCGAG)*

The primer OPAW 05 gave a good amplification in the all the 17 accession tested. There were a total of 39 polymorphic and without any monomorphic bands. Some notable specific bands were observed in some accessions, which are absent in all other accessions (Plate 18).

OPAW 12*(GAGCAAGGCA)*

The primer OPAW 12 gave a good amplification in the all the 17 accession tested. There were a total of 34 polymorphic and without any monomorphic bands. Some notable specific bands were observed in some accessions, which are absent in all other accessions (Plate 19).

OPBA 03 *(GTGCGAGAAC)*

The primer OPBA 03 gave a good amplification in the all the 16 accession tested There were a total of 46 polymorphic and without any monomorphic bands. Except in lane 13. (*Rhynchostylis retusa*) Some notable specific bands were observed in some accessions, which are absent in all other accessions (Plate 20).

OPE 12*(TTATCGCCCC)*

The primer OPE 12 gave the amplification in the all the 17 accession tested. There were a total of 49 polymorphic and without any monomorphic bands. Some notable specific bands were observed in some accessions, which are absent in all other accessions (Plate 21).

OPM 15 *(GACCTACCAC)*

The primer OPM 15 gave a good amplification in the all the 17 accession tested. There were a total of 36 polymorphic and without any monomorphic bands. Some notable specific bands were observed in some accessions, which are absent in all other accessions (Plate 22).

Table 20. Total number of bands amplified with primers.

Sl. No.	NAME	OPA 05	OPA 11	OPA 16	OPA 18	OPA 20	OPAW 05	OPAW 12	OPBA 03	OPE 12	OPM 15
1	*Vanda testacea*	2	4	4	4	2	5	4	2	3	5
2	*Cymbidium* 1	2	4	4	5	3	1	2	4	3	3
3	*Cymbidium* 2	5	3	3	5	4	5	4	3	6	3
4	*Cymbidium* 3	1	3	3	4	1	3	1	6	1	3
5	*Bulbophyllum elongatum*	4	2	3	2	2	1	2	3	3	1
6	*Bulbophyllum fischeri*	2	2	1	2	2	1	2	4	3	1
7	*Phalenopsis* hybrid	3	3	4	1	2	3	2	2	2	1
8	*Oncidium tolumina*	3	3	2	1	1	4	2	3	4	2
9	*Dendrobium* hybrid	3	3	4	2	3	1	2	4	2	4
10	*Cattleya*	1	1	2	1	2	4	1	2	2	1
11	*Aerides maculosa*	1	2	1	1	3	1	1	3	5	1
12	*Pholidota imbricata*	1	0	2	2	1	1	1	3	4	3
13	*Rhynchostylis retusa*	1	2	0	1	1	2	1	0	1	1
14	*Flickingeria nodosa*	2	2	3	4	2	2	3	4	4	2
15	*Acampe praemorsa*	2	2	1	4	2	2	3	1	2	2
16	Mini *Cattleya*	3	4	1	1	3	2	2	1	2	1
17	*Coelogyne breviscapa*	1	3	1	1	1	1	1	1	2	2

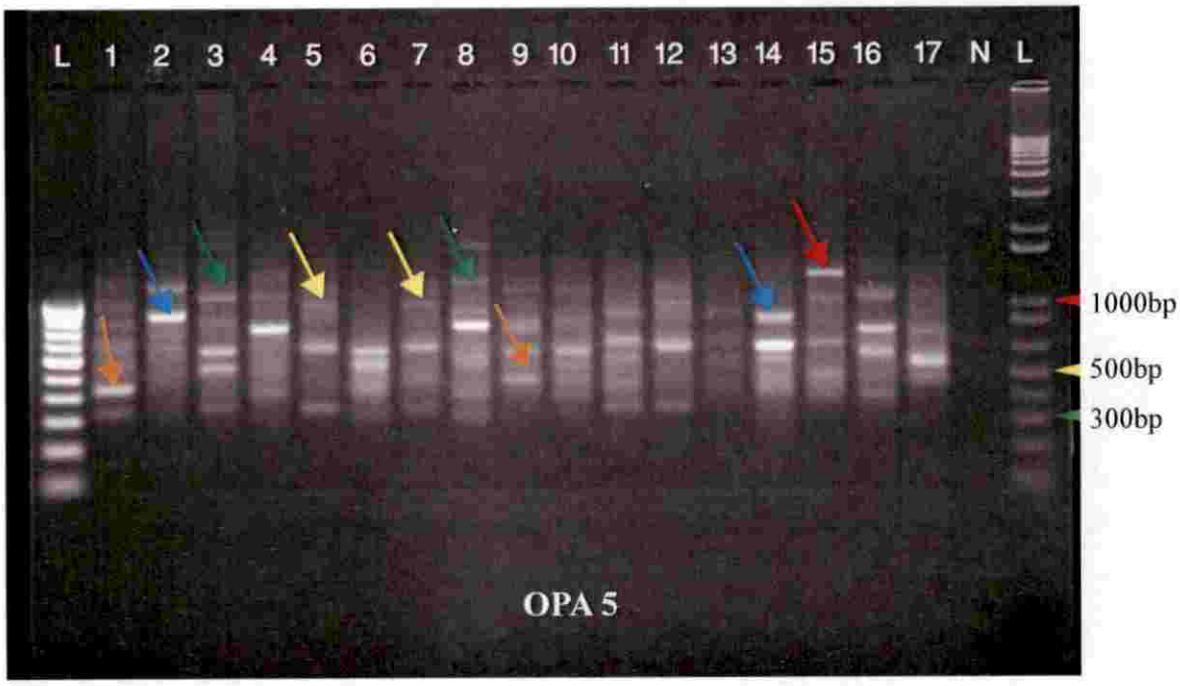


Plate 13: RAPD banding pattern of orchids with primer OPA 5.

LL	100 base pair ladder	10	*Cattleya*
1	*Vanda testacea*	11	*Aeridis maculosa*
2	*Cymbidium* 1	12	*Pholidota imbricata*
3	*Cymbidium* 2	13	*Rhynchostylis retusa*
4	*Cymbidium* 3	14	*Flickingeria nodosa*
5	*Bulbophyllum elongatum*	15	*Acampe praemorsa*
6	*Bulbophyllum fischeri*	16	Mini *Cattleya*
7	*Phalenopsis* hybrid	17	*Coelogyne breviscapa*
8	*Oncidium tolumina*	N	Negative control
9	*Dendrobium* hybrid 6	LR	1 kilo base pair ladder

Species specific bands detected using OPA 5		
Band size (bp)	**Lane number**	**Colour code of the arrow in Plate 13**
1500	15	Red
1700	3, 8	Green
1000	5, 7	Yellow
900	2, 14	Blue
450	1, 9	Orange

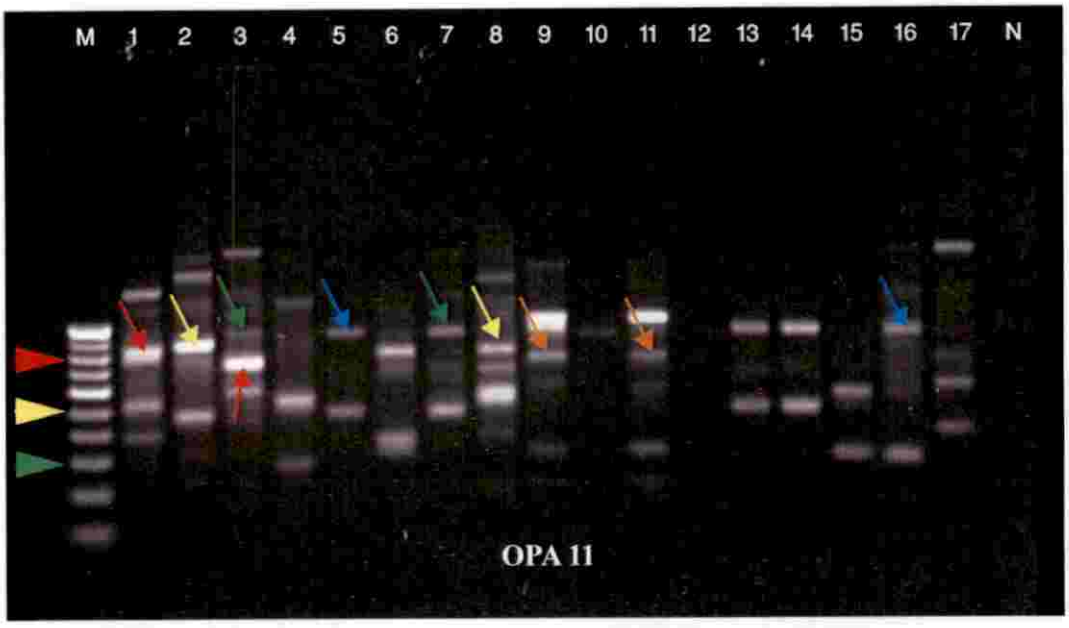


Plate 14: RAPD banding pattern of orchids with primer OPA 11.

LL	100 base pair ladder	10	*Cattleya*
1	*Vanda testacea*	11	*Aeridis maculosa*
2	*Cymbidium* 1	12	*Pholidota imbricata*
3	*Cymbidium* 2	13	*Rhynchostylis retusa*
4	*Cymbidium* 3	14	*Flickingeria nodosa*
5	*Bulbophyllum elongatum*	15	*Acampe praemorsa*
6	*Bulbophyllum fischeri*	16	Mini *Cattleya*
7	*Phalenopsis* hybrid	17	*Coelogyne breviscapa*
8	*Oncidium tolumina*	N	Negative control
9	*Dendrobium* hybrid 6		

Species specific bands detected using OPA 11		
Band size (bp)	**Lane number**	**Colour code of the arrow in Plate 14**
1450, 900	1, 2	Red
1600	2, 7	Green
1500	2, 8	Yellow
1400	4, 6	Blue
1100	9, 11	Orange

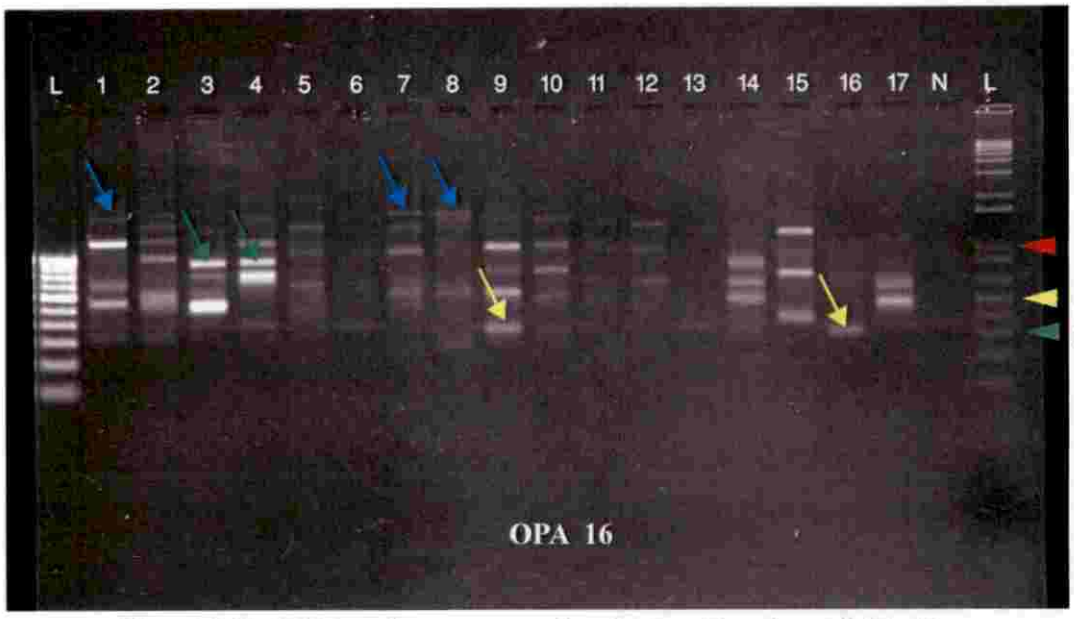


Plate 15: RAPD banding pattern of orchids with primer OPA 16.

LL	100 base pair ladder	10	*Cattleya*
1	*Vanda testacea*	11	*Aeridis maculosa*
2	*Cymbidium* 1	12	*Pholidota imbricata*
3	*Cymbidium* 2	13	*Rhynchostylis retusa*
4	*Cymbidium* 3	14	*Flickingeria nodosa*
5	*Bulbophyllum elongatum*	15	*Acampe praemorsa*
6	*Bulbophyllum fischeri*	16	Mini *Cattleya*
7	*Phalenopsis* hybrid	17	*Coelogyne breviscapa*
8	*Oncidium tolumina*	N	Negative control
9	*Dendrobium* hybrid 6	LR	1 kilo base pair ladder

Species specific bands detected using OPA 16		
Band size (bp)	**Lane number**	**Colour code of the arrow in Plate 15**
950	3, 4	Green
400	9, 16	Yellow
1650	1, 7, 8	Blue

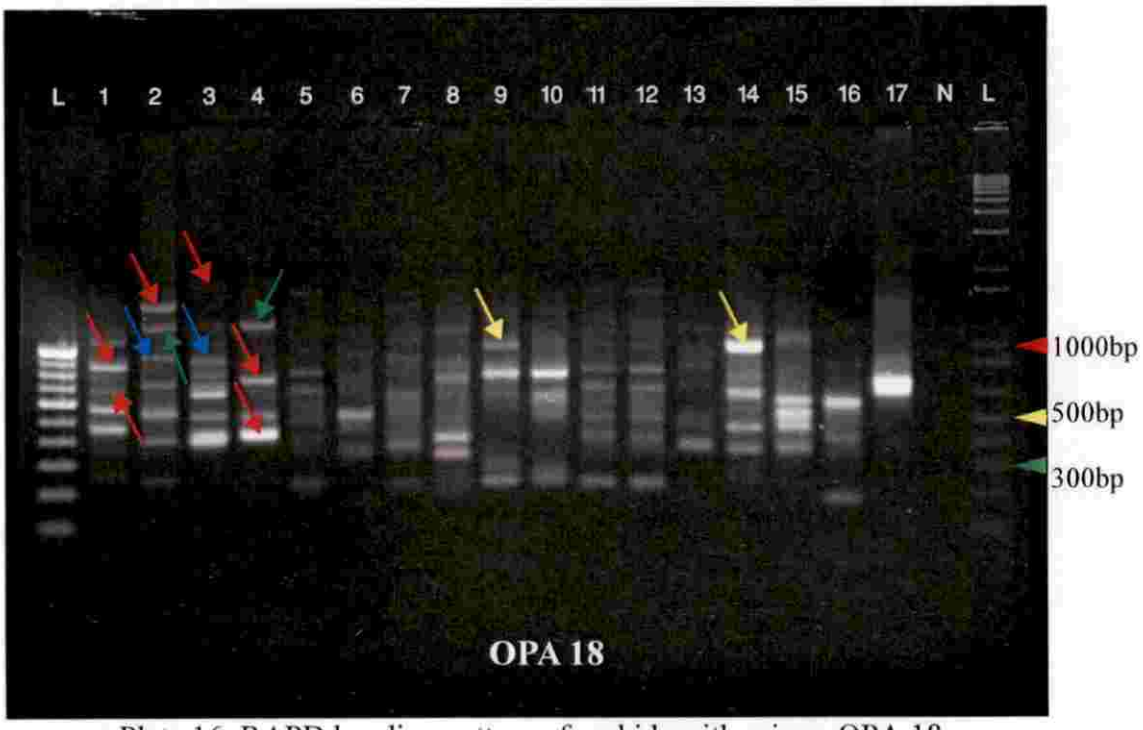


Plate 16: RAPD banding pattern of orchids with primer OPA 18.

LL	100 base pair ladder	10	*Cattleya*
1	*Vanda testacea*	11	*Aeridis maculosa*
2	*Cymbidium* 1	12	*Pholidota imbricata*
3	*Cymbidium* 2	13	*Rhynchostylis retusa*
4	*Cymbidium* 3	14	*Flickingeria nodosa*
5	*Bulbophyllum elongatum*	15	*Acampe praemorsa*
6	*Bulbophyllum fischeri*	16	Mini *Cattleya*
7	*Phalenopsis* hybrid	17	*Coelogyne breviscapa*
8	*Oncidium tolumina*	N	Negative control
9	*Dendrobium* hybrid 6	LR	1 kilo base pair ladder

Species specific bands detected using OPA 18		
Band size (bp)	**Lane number**	**Colour code of the arrow in Plate 16**
1650, 1500, 1100, 890, 750, 400	3, 2, 1, 1, 4, 4	Red
1400	2, 4	Green
1000	9, 14	Yellow
900	2, 3	Blue

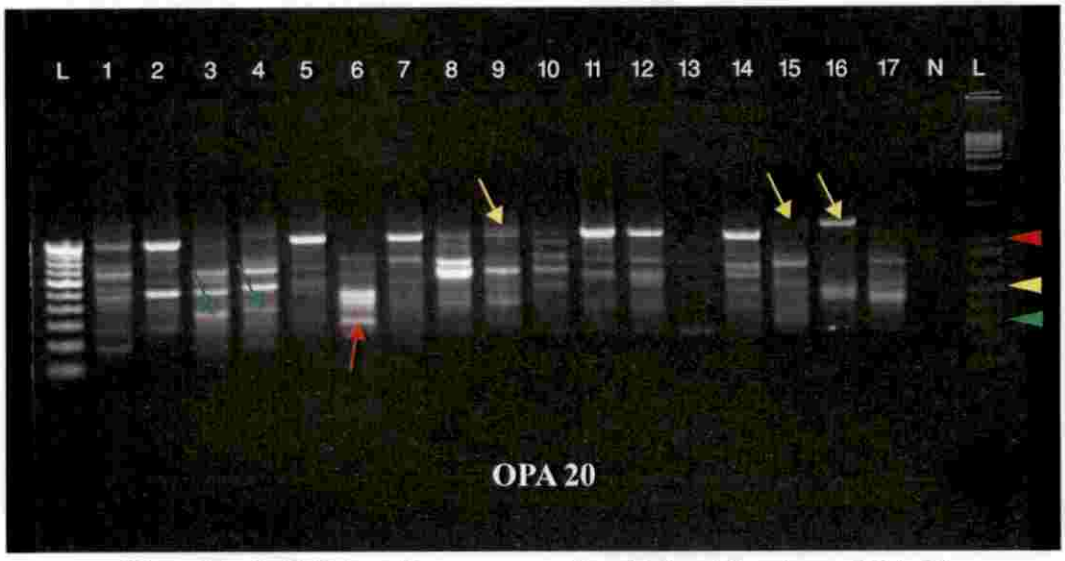


Plate 17: RAPD banding pattern of orchids with primer OPA 20

LL	100 base pair ladder	10	*Cattleya*
1	*Vanda testacea*	11	*Aeridis maculosa*
2	*Cymbidium* 1	12	*Pholidota imbricata*
3	*Cymbidium* 2	13	*Rhynchostylis retusa*
4	*Cymbidium* 3	14	*Flickingeria nodosa*
5	*Bulbophyllum elongatum*	15	*Acampe praemorsa*
6	*Bulbophyllum fischeri*	16	Mini *Cattleya*
7	*Phalenopsis* hybrid	17	*Coelogyne breviscapa*
8	*Oncidium tolumina*	N	Negative control
9	*Dendrobium* hybrid 6	LR	1 kilo base pair ladder

Species specific bands detected using OPA 20		
Band size (bp)	**Lane number**	**Colour code of the arrow in Plate 17**
300	6	Red
350	3,4	Green
1300	9,15,16	Yellow

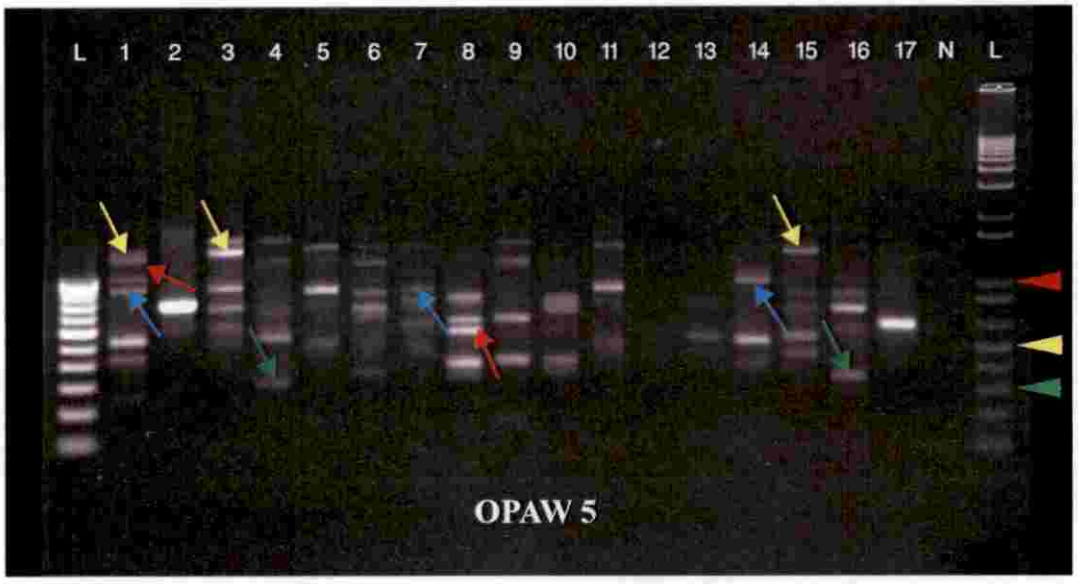


Plate 18: RAPD banding pattern of orchids with primer OPAW 5

LL	100 base pair ladder	10	*Cattleya*
1	*Vanda testacea*	11	*Aeridis maculosa*
2	*Cymbidium* 1	12	*Pholidota imbricata*
3	*Cymbidium* 2	13	*Rhynchostylis retusa*
4	*Cymbidium* 3	14	*Flickingeria nodosa*
5	*Bulbophyllum elongatum*	15	*Acampe praemorsa*
6	*Bulbophyllum fischeri*	16	Mini *Cattleya*
7	*Phalenopsis* hybrid	17	*Coelogyne breviscapa*
8	*Oncidium tolumina*	N	Negative control
9	*Dendrobium* hybrid 6	LR	1 kilo base pair ladder

Species specific bands detected using OPAW 5		
Band size (bp)	**Lane number**	**Colour code of the arrow in Plate 18**
1590, 560	1, 8	Red
300	4, 16	Green
1600	1, 3, 15	Yellow
1000	1, 7, 14	Blue

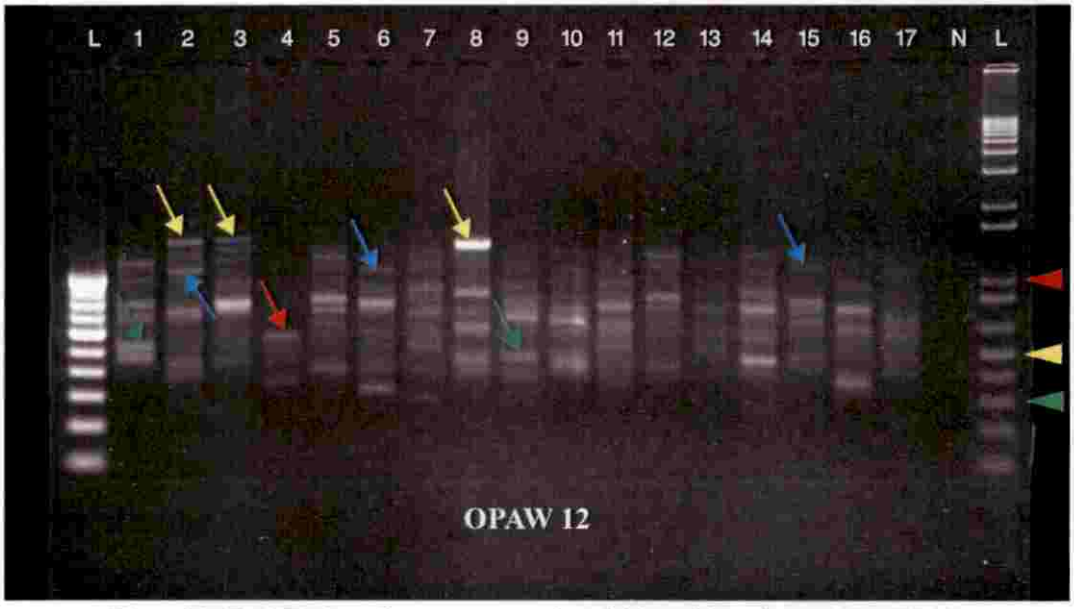


Plate 19: RAPD banding pattern of orchids with primer OPAW 12

LL	100 base pair ladder	10	*Cattleya*
1	*Vanda testacea*	11	*Aeridis maculosa*
2	*Cymbidium* 1	12	*Pholidota imbricata*
3	*Cymbidium* 2	13	*Rhynchostylis retusa*
4	*Cymbidium* 3	14	*Flickingeria nodosa*
5	*Bulbophyllum elongatum*	15	*Acampe praemorsa*
6	*Bulbophyllum fischeri*	16	Mini *Cattleya*
7	*Phalenopsis* hybrid	17	*Coelogyne breviscapa*
8	*Oncidium tolumina*	N	Negative control
9	*Dendrobium* hybrid 6	LR	1 kilo base pair ladder

Species specific bands detected using OPAW 12		
Band size (bp)	**Lane number**	**Colour code of the arrow in Plate 19**
600	4	Red
550	1, 9	Green
1650	2, 3, 8	Yellow
1200	2, 6, 15	Blue

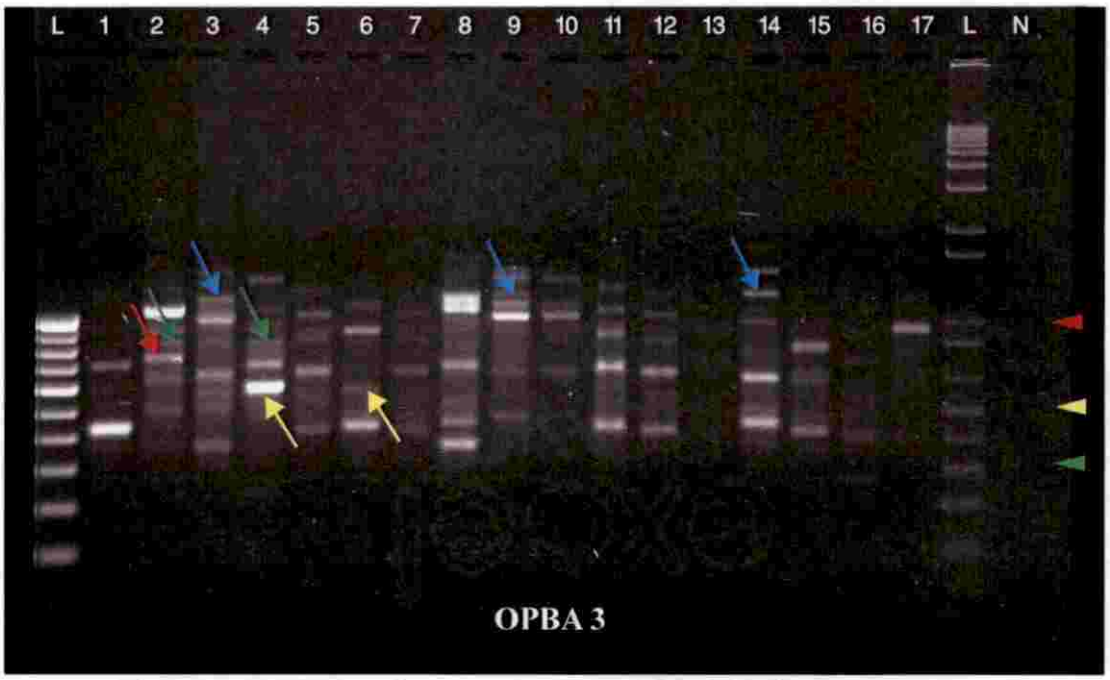


Plate 20: RAPD banding pattern of orchids with primer OPBA 3

LL	100 base pair ladder	10	*Cattleya*
1	*Vanda testacea*	11	*Aeridis maculosa*
2	*Cymbidium* 1	12	*Pholidota imbricata*
3	*Cymbidium* 2	13	*Rhynchostylis retusa*
4	*Cymbidium* 3	14	*Flickingeria nodosa*
5	*Bulbophyllum elongatum*	15	*Acampe praemorsa*
6	*Bulbophyllum fischeri*	16	Mini *Cattleya*
7	*Phalenopsis* hybrid	17	*Coelogyne breviscapa*
8	*Oncidium tolumina*	LR	1 kilo base pair ladder
9	*Dendrobium* hybrid 6	N	Negative control

Species specific bands detected using OPBA 3		
Band size(bp)	**Lane number**	**Colour code of the arrow in Plate 20**
800	2	Red
850	2, 4	Green
600	4, 6	Yellow
1600	3, 9, 14	Blue

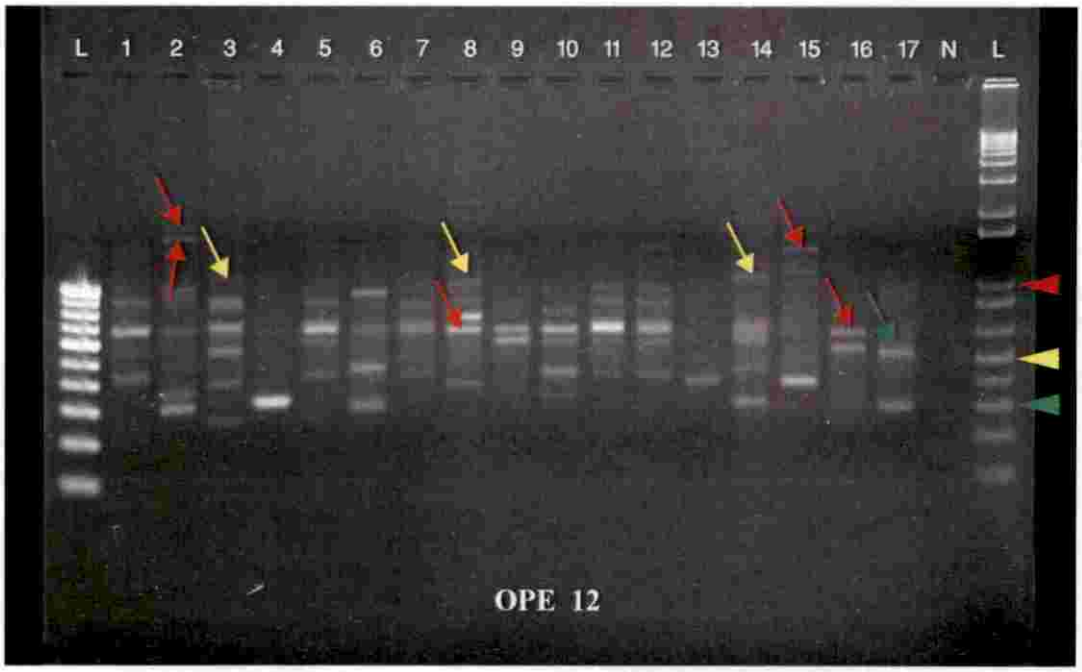


Plate 21: RAPD banding pattern of orchids with primer OPE 12.

LL	100 base pair ladder	10	*Cattleya*
1	*Vanda testacea*	11	*Aeridis maculosa*
2	*Cymbidium* 1	12	*Pholidota imbricata*
3	*Cymbidium* 2	13	*Rhynchostylis retusa*
4	*Cymbidium* 3	14	*Flickingeria nodosa*
5	*Bulbophyllum elongatum*	15	*Acampe praemorsa*
6	*Bulbophyllum fischeri*	16	Mini *Cattleya*
7	*Phalenopsis* hybrid	17	*Coelogyne breviscapa*
8	*Oncidium tolumina*	N	Negative control
9	*Dendrobium* hybrid 6	LR	1 kilo base pair ladder

Species specific bands detected using OPE 12		
Band size (bp)	**Lane number**	**Colour code of the arrow in Plate 21**
1650,1600,1550,800,600	2,2,15,8,16	Red
590	3,17	Green
1100	3,8,14	Yellow

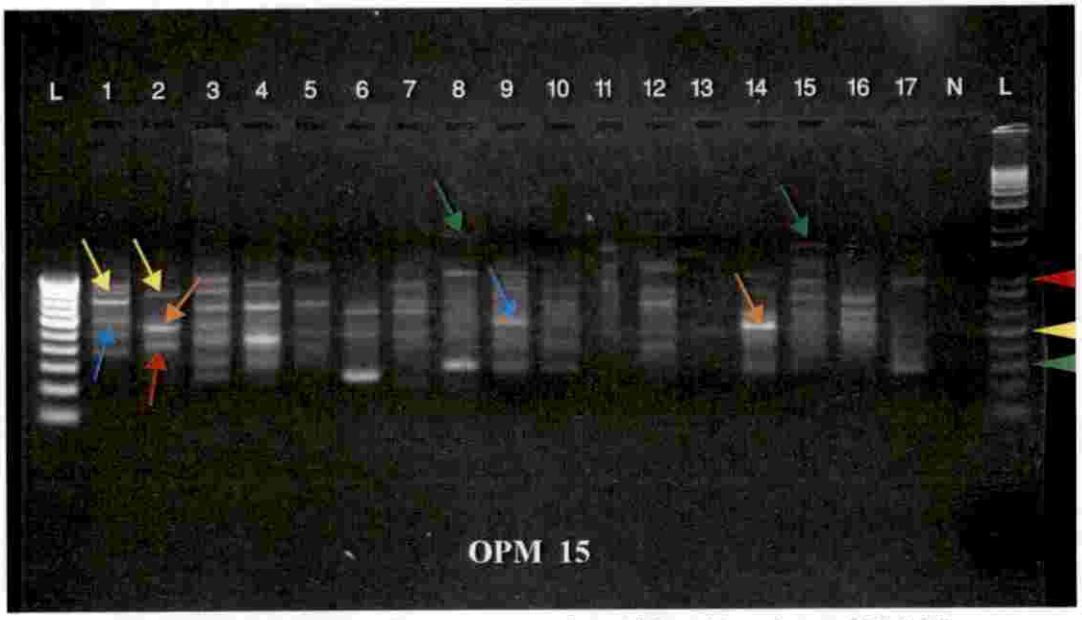


Plate 22: RAPD banding pattern of orchids with primer OPM 15

LL	100 base pair ladder	10	*Cattleya*
1	*Vanda testacea*	11	*Aeridis maculosa*
2	*Cymbidium* 1	12	*Pholidota imbricata*
3	*Cymbidium* 2	13	*Rhynchostylis retusa*
4	*Cymbidium* 3	14	*Flickingeria nodosa*
5	*Bulbophyllum elongatum*	15	*Acampe praemorsa*
6	*Bulbophyllum fischeri*	16	Mini *Cattleya*
7	*Phalenopsis* hybrid	17	*Coelogyne breviscapa*
8	*Oncidium tolumina*	N	Negative control
9	*Dendrobium* hybrid 6	LR	1 kilo base pair ladder

Species specific bands detected using OPM 15		
Band size (bp)	**Lane number**	**Colour code of the arrow in Plate 22**
400	2	Red
1700	8, 15	Green
900	1, 2	Yellow
600	1, 9	Blue
500	2, 14	Orange

4.5.4.1 Diversity analysis:

Extent of diversity was estimated based on analysis of 17 accessions of orchids using 10 RAPD primers. The number of bands varied from 35 (OPA 20) to 49 (OPE-12) with average of 23.47 bands per assay unit. For separate assay units, PIC values ranged from 0.23 (OPA-18) to 0.34 (OPA-16) (Table 21).The PCR amplification of template DNA produced a total of 399 RAPD bands for the genetic relationship study, only distinct, reproducible, well resolved amplicons in the size range of 300 – 1700 bp were scored as present (1) or absent (0) from the images of the gels. From band scores, a binary data matrix was constructed and genetic similarities were calculated among all possible pairs of accessions. A dendrogram of genetic relationship was produced by clustering the data with SIMQUAL of the Numerical taxonomy and Multivariant Analysis System program Package for PC (NTSYS-pc ver. 2.021i Package) (Rohlf, 1999).

Cluster analysis was used to group of the accessions to construct dendrograms based on RAPD markers. The dendrogram separated the 17 accessions into 6 groups (Plate 23).

Group 1 : PDK/ORP-1 (*Vanda testacea*), PDK/ORP-12 (*Oncidium tolumina*).

Group 2 : PDK/ORP-29 (*Bulbophyllum elongatum),* PDK/ORP-32 (*Pholidota imbricata*), PDK/ORP-30 (*Aerides maculosa),* PDK/ORP-10 (*Phalenopsis* hybrid), PDK/ORP-18 (*Dendrobium* hybrid *6),* PDK/ORP-22 (*Cattleya),* and PDK/ORP-39 (*Flickingeria nodosa).*

Group 3 : PDK/ORP-24 (*Cymbidium 2),* PDK/ORP-28 (*Coelogyne breviscapa).*

Group 4 : PDK/ORP-35 (*Cymbidium 3)*, PDK/ORP-45 (*Acampe praemorsa),* PDK/ORP-14 (Mini *Cattleya).*

Group 5 : PDK/ORP-7 (*Cymbidium* 1*)*, PDK/ORP-31 (*Bulbophyllum fischeri).*

Group 6 : PDK/ORP-33 (*Rhynchostylis retusa*).

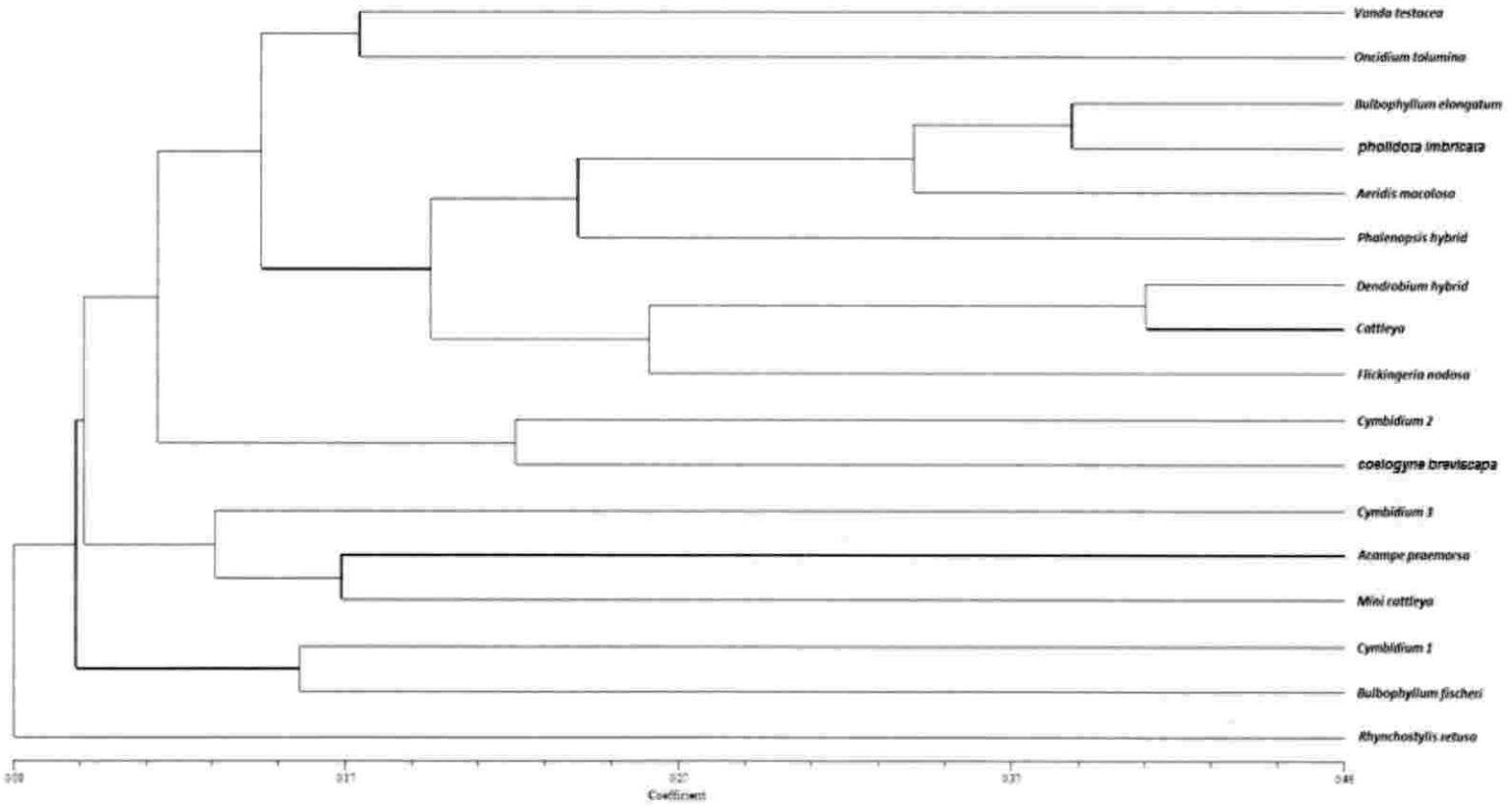


Plate 23: Phylogenetic tree for 17 accessions based on RAPD markers

Table 21. Primers with number of diagnostic bands for species and PIC value.

Sl. No.	Primer Name	No of Diagnostic Bands	PIC
1	OPA 05	1	0.28
2	OPA 11	2	0.25
3	OPA 16	0	0.34
4	OPA 18	6	0.23
5	OPA 20	1	0.33
6	OPAW 05	2	0.28
7	OPAW 12	1	0.32
8	OPBA 03	1	0.32
9	OPE 12	5	0.25
10	OPM 15	1	0.28

4.5.5 RAPD profiling for detecting intra generic polymorphism

In the 2nd phase of the experiment, RAPD analysis was conducted focusing on the intra generic diversity of *Vanda* and *Dendrobium* species. A total of 9 accessions from *Vanda* and 9 accessions from *Dendrobium* were amplified with 5 primers which had been amplified properly in the previous experiment. The reaction mixture and the condition used for the experiment were same as mentioned before.

4.5.5.1 RAPD profiling of Vanda accessions

The accessions were selected in such a way that they are from the different parts of Central Western Ghats (Table 22). A high level of diversity is found within the accessions tested. However there was monomorphism in some of the accessions tested of *Vanda*.

Table 22. *Vanda* accessions selected for detecting intra generic polymorphism.

Sl. No.	Accession code	Name of natural habitat
1	PDK/ORP-1	Nagarhole
2	PDK/ORP-2	Nagarhole
3	PDK/ORP-3	Nagarhole
4	PDK/ORP-5	Thithmathi
5	PDK/ORP-19	Sullia
6	PDK/ORP-21	Nagarhole
7	PDK/ORP-23	Thithmathi
8	PDK/ORP-36	Bharamagiri
9	PDK/ORP-46	Nagarhole

OPA 05 *(AGGGGTCTTG)*

The primer OPA 5 gave a good amplification in all the 9 accessions tested. There were a total of 27 polymorphic and without any monomorphic bands. Some notable specific bands present in one accession were absent in all the other accessions. (Plate 24).

OPA 18 *(AGGTGACCGT)*

The primer OPA 18 gave a good amplification in all the 9 accessions tested. There were a total of 30 polymorphic and without any monomorphic bands. The primer was efficient and some notable specific bands were present in the accession which are absent in all the other accessions (Plate 25).

OPBA 03 *(GTGCGAGAAC)*

The primer OPBA 3 gave a good amplification in all the 9 accessions tested. There were a total of 53 polymorphic and 1 monomorphic bands. Some notable specific bands were present in the accession which are absent in all the other accessions (Plate 26).

OPE 12 *(TTATCGCCCC)*

The primer OPE 12 gave a good amplification in the all the 9 accession tested. There were a total of 31 polymorphic and without any monomorphic bands. The primer was efficient and some notable specific bands were present in the accession which are absent in all the other accessions (Plate 27).

OPM 15 *(GACCTACCAC)*

The primer OPM 15 gave a good amplification in the all the 9 accession tested. There were a total of 51 polymorphic and without any monomorphic bands. The primer was efficient and some notable specific bands were present in the accession which are absent in all the other accessions (Plate 28).

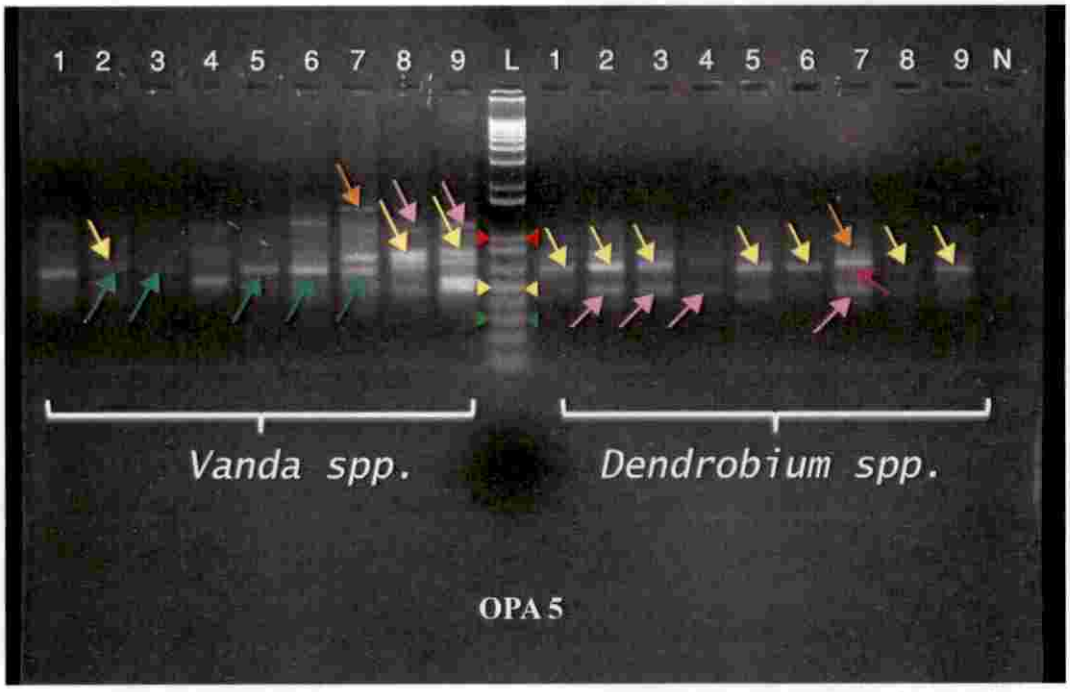


Plate 24: RAPD banding pattern of *Vanda* and *Dendrobium* accessions using primer OPA 5.

1	PDK/ORP-1	1	PDK/ORP-8
2	PDK/ORP-2	2	PDK/ORP-11
3	PDK/ORP-3	3	PDK/ORP-13
4	PDK/ORP-5	4	PDK/ORP-15
5	PDK/ORP-19	5	PDK/ORP-16
6	PDK/ORP-21	6	PDK/ORP-17
7	PDK/ORP-23	7	PDK/ORP-18
8	PDK/ORP-36	8	PDK/ORP-20
9	PDK/ORP-46	9	PDK/ORP-34
L	1 kilo base pair ladder	N	Negative control

On ladder (L), green colour triangle: 300 base pair; yellow colour triangle: 500 base pair; red colour triangle: 1000 base pair.

Specific bands for *Vanda* species		
Band size (bp)	**Lane number**	**Colour code of the arrow in Plate 24**
1400	7	Orange
1050	8, 9	Pink
700	2, 8, 9	Yellow
550	2, 3, 5, 6, 7, 9	Green
Specific bands for *Dendrobium* species		
Band size (bp)	**Lane number**	**Colour code of the arrow in Plate 24**
750, 400	7, 5	Orange
450	2, 3, 4, 7	Pink
650	1, 2, 3, 5, 6, 7, 8, 9	Yellow

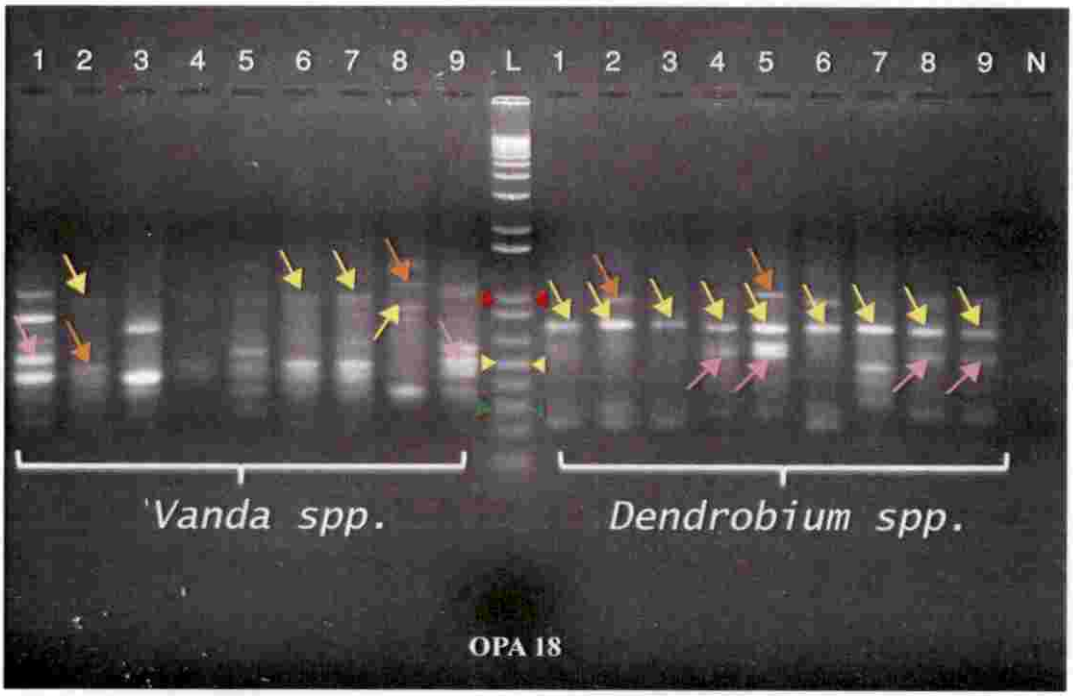


Plate 25: RAPD banding pattern of *Vanda* and *Dendrobium* accessions using primer OPA 18.

1	PDK/ORP-1	1	PDK/ORP-8
2	PDK/ORP-2	2	PDK/ORP-11
3	PDK/ORP-3	3	PDK/ORP-13
4	PDK/ORP-5	4	PDK/ORP-15
5	PDK/ORP-19	5	PDK/ORP-16
6	PDK/ORP-21	6	PDK/ORP-17
7	PDK/ORP-23	7	PDK/ORP-18
8	PDK/ORP-36	8	PDK/ORP-20
9	PDK/ORP-46	9	PDK/ORP-34
L	1 kilo base pair ladder	N	Negative control

On ladder (L), green colour triangle: 300 base pair; yellow colour triangle: 500 base pair; red colour triangle: 1000 base pair.

Specific bands for *Vanda* species		
Band size (bp)	**Lane number**	**Colour code of the arrow in Plate 25**
1200, 450	8, 2	Orange
500	1, 9	Pink
1000	2, 6, 7, 8	Yellow
Specific bands for *Dendrobium* species		
Band size (bp)	**Lane number**	**Colour code of the arrow in Plate 25**
1000, 950	5, 2	Orange
525, 500	4, 5 and 8, 9	Pink
700	1, 2, 3, 4, 5, 6, 7, 8, 9	Yellow

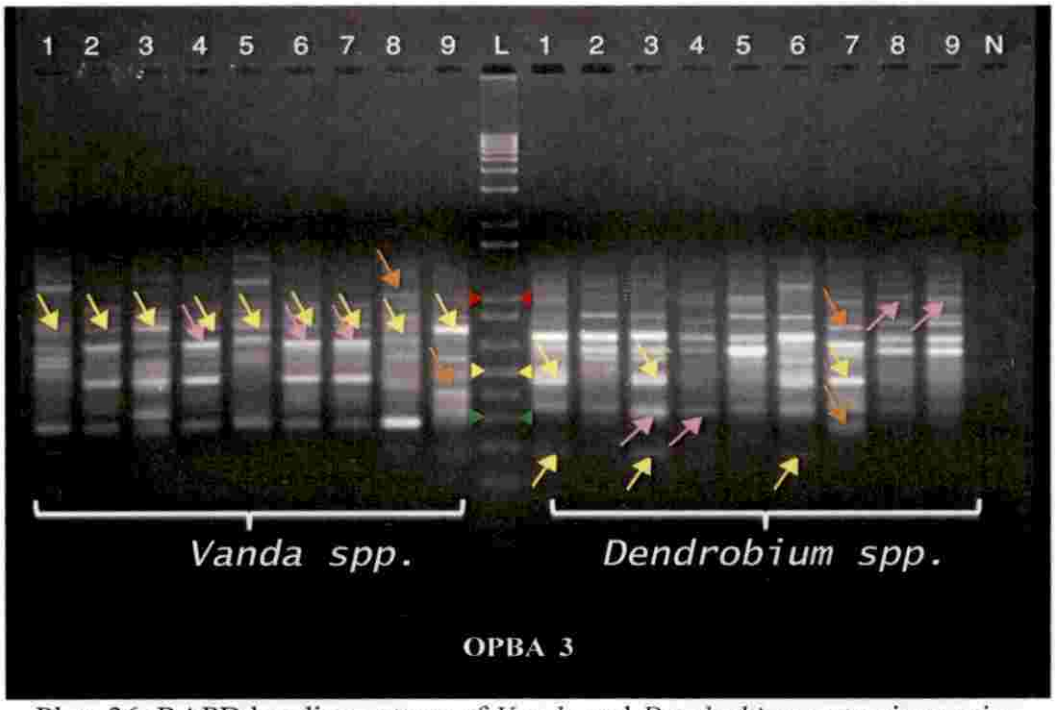


Plate 26: RAPD banding pattern of *Vanda* and *Dendrobium* accessions using primer OPBA 3.

1	PDK/ORP-1	1	PDK/ORP-8
2	PDK/ORP-2	2	PDK/ORP-11
3	PDK/ORP-3	3	PDK/ORP-13
4	PDK/ORP-5	4	PDK/ORP-15
5	PDK/ORP-19	5	PDK/ORP-16
6	PDK/ORP-21	6	PDK/ORP-17
7	PDK/ORP-23	7	PDK/ORP-18
8	PDK/ORP-36	8	PDK/ORP-20
9	PDK/ORP-46	9	PDK/ORP-34
L	1 kilo base pair ladder	N	Negative control

On ladder (L), green colour triangle: 300 base pair; yellow colour triangle: 500 base pair; red colour triangle: 1000 base pair.

Specific bands for *Vanda* species		
Band size (bp)	**Lane number**	**Colour code of the arrow in Plate 26**
1000, 400	8, 9	Orange
650	4, 6, 7	Pink
700	1, 2, 3, 4, 5, 6, 7, 8, 9	Yellow
Specific bands for *Dendrobium* species		
Band size (bp)	**Lane number**	**Colour code of the arrow in Plate 26**
780, 250	7	Orange
800, 300	8, 9 and 3, 4	Pink
450, 175	1, 3, 7 and 1, 3, 6	Yellow

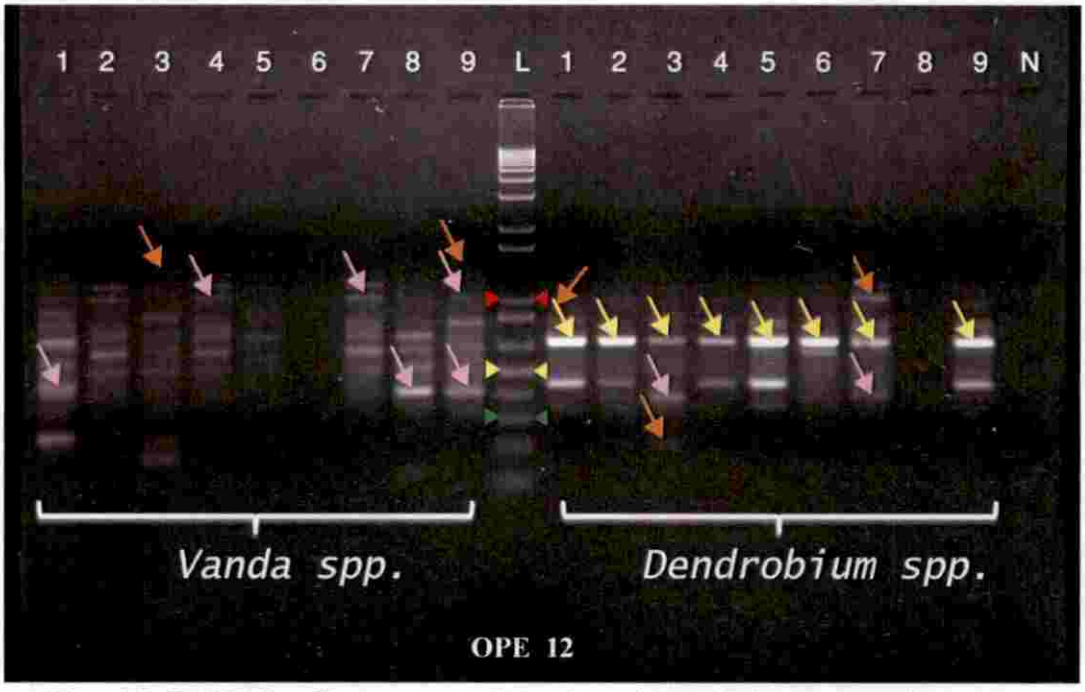


Plate 27: RAPD banding pattern of *Vanda* and *Dendrobium* accessions using primer OPE 12.

1	PDK/ORP-1	1	PDK/ORP-8
2	PDK/ORP-2	2	PDK/ORP-11
3	PDK/ORP-3	3	PDK/ORP-13
4	PDK/ORP-5	4	PDK/ORP-15
5	PDK/ORP-19	5	PDK/ORP-16
6	PDK/ORP-21	6	PDK/ORP-17
7	PDK/ORP-23	7	PDK/ORP-18
8	PDK/ORP-36	8	PDK/ORP-20
9	PDK/ORP-46	9	PDK/ORP-34
L	1 kilo base pair ladder	N	Negative control

On ladder (L), green colour triangle: 300 base pair; yellow colour triangle: 500 base pair; red colour triangle: 1000 base pair.

Specific bands for *Vanda* species		
Band size (bp)	**Lane number**	**Colour code of the arrow in Plate 27**
1400, 1200	9, 3	Orange
1000, 400	4, 7, 9 and 1, 8, 9	Pink
Specific bands for *Dendrobium* species		
Band size (bp)	**Lane number**	**Colour code of the arrow in Plate 27**
1000, 800, 200	7, 1, 3	Orange
350	3, 7	Pink
650	1, 2, 3, 4, 5, 6, 7, 9	Yellow

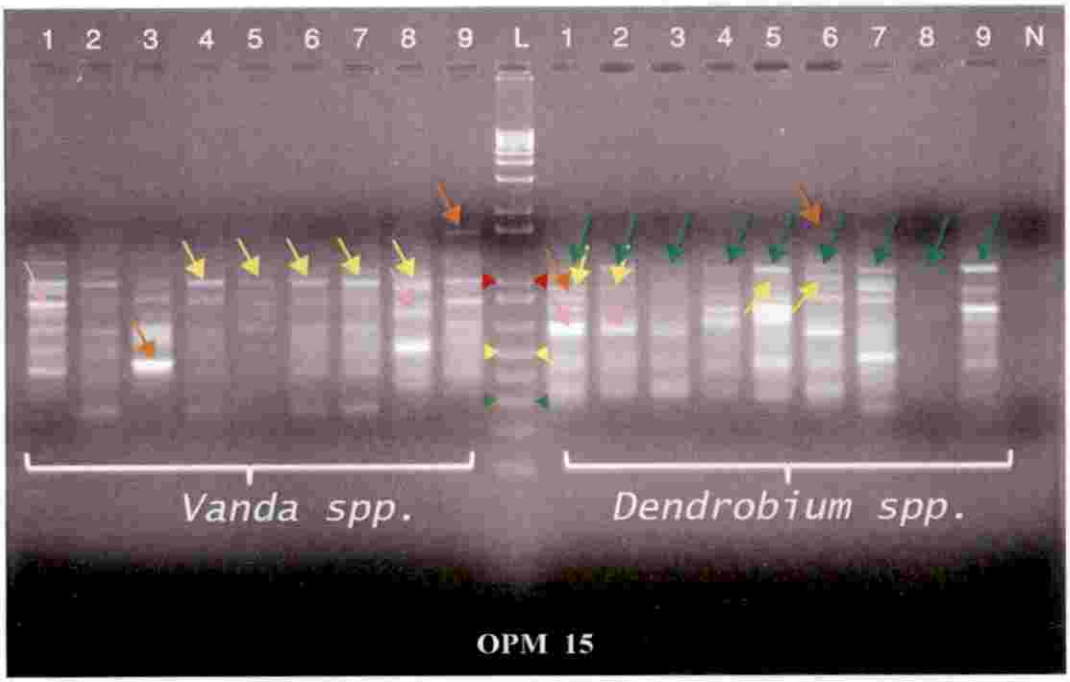


Plate 28: RAPD banding pattern of *Vanda* and *Dendrobium* accessions using primer OPM 15

1	PDK/ORP-1	1	PDK/ORP-8
2	PDK/ORP-2	2	PDK/ORP-11
3	PDK/ORP-3	3	PDK/ORP-13
4	PDK/ORP-5	4	PDK/ORP-15
5	PDK/ORP-19	5	PDK/ORP-16
6	PDK/ORP-21	6	PDK/ORP-17
7	PDK/ORP-23	7	PDK/ORP-18
8	PDK/ORP-36	8	PDK/ORP-20
9	PDK/ORP-46	9	PDK/ORP-34
L	1 kilo base pair ladder	N	Negative control

On ladder (L), green colour triangle: 300 base pair; yellow colour triangle: 500 base pair; red colour triangle: 1000 base pair.

Specific bands for *Vanda* species		
Band size (bp)	**Lane number**	**Colour code of the arrow in Plate 28**
1625, 425	9, 3	Orange
750	1, 8	Pink
975	4, 5, 6, 7, 8	Yellow
Specific bands for *Dendrobium* species		
Band size (bp)	**Lane number**	**Colour code of the arrow in Plate 28**
1625, 850, 500	6, 1, 7	Orange
650	1, 2	Pink
900	1, 2, 5, 6	Yellow
1100	1, 2, 3, 4, 5, 7, 8, 9	Green

4.5.5.2 RAPD profiling of Dendrobium accessions

The accessions were selected in such a way that they are from the different parts of Central Western Ghats (Table 23). A high level of diversity is found within the accessions tested. However there was monomorphism in some of the accession tested

Table 23. *Dendrobium* accessions selected for detecting intra generic polymorphism.

Sl. No.	Accession code	Name of natural habitat
1	PDK/ORP-8	Padannakkad
2	PDK/ORP-11	Bengaluru
3	PDK/ORP-13	Bengaluru
4	PDK/ORP-15	Bengaluru
5	PDK/ORP-16	Bengaluru
6	PDK/ORP-17	Bengaluru
7	PDK/ORP-18	Bengaluru
8	PDK/ORP-20	Bharamagiri
9	PDK/ORP-34	Bharamagiri

OPA 05 *(AGGGGTCTTG)*

The primer OPA 5 gave a good amplification in the all the 9 accession tested. There were a total of 21 polymorphic and without any monomorphic bands. Some notable specific bands were present in the accession which are absent in all the other accessions (Plate 24).

OPA 18 *(AGGTGACCGT)*

The primer OPA 18 gave a good amplification in the all the 9 accession tested. There were a total of 31 polymorphic and 1 monomorphic bands. The primer was efficient and some notable specific bands were present in the accession which are absent in all the other accessions (Plate 25).

OPBA 03 *(GTGCGAGAAC)*

The primer OPBA 3 gave a good amplification in the all the 9 accession tested. There were a total of 61 polymorphic and without any monomorphic

bands. Some notable specific bands were present in the accession which are absent in all the other accessions (Plate 26).

***OPE 12** (TTATCGCCCC)*

The primer OPE 12 gave a good amplification in the all the 9 accession tested. There were a total of 22 polymorphic and without any monomorphic bands. The primer was efficient and some notable specific bands were present in the accession which are absent in all the other accessions (Plate 27).

***OPM 15** (GACCTACCAC)*

The primer OPM 15 gave a good amplification in the all the 9 accession tested. There were a total of 51 polymorphic and without any monomorphic bands. The primer was efficient and some notable specific bands were present in the accession which are absent in all the other accessions (Plate 28).

4.5.5.3 Intra-generic diversity analysis

Extent of diversity was estimated based on analysis of 9 accessions of *Vanda* and *Dendrobium* by using 5 RAPD primers. The number of bands varied from 27 (OPA 5) to 53 (OPBA-3) with average of 21.33 bands per assay unit in *Vanda* and 21 (OPA 5) to 61 (OPBA-3) with average of 20.66 bands per assay unit in. For separate assay units, PIC values ranged from 0.23 (OPA-18) to 0.32 (OPBA-3) (Table 21).The PCR amplification of template DNA produced a total of 192 RAPD bands in *Vanda* where as 186 RAPD bands in *Dendrobium*. In the genetic relationship study only distinct, reproducible, well resolved amplicon range of 150–1450 bp in Vanda and 175–1625 bp in *Dendrobium* were scored as present (1) or absent (0) from the images of the gels. From band scores, a binary data matrix was constructed and genetic similarities were calculated among all possible pairs of accessions as in inter-generic analysis.

Vanda Cluster analyses

Cluster analysis was used to group *Vanda* accessions to construct dendrograms based on RAPD markers. The dendrogram separated the 9 accessions into 3 groups (Plate 29).

Group 1 : PDK/ORP-01, PDK/ORP-03

Group 2 : PDK/ORP-05, PDK/ORP-21, PDK/ORP-23, PDK/ORP-19, PDK/ORP-36, PDK/ORP-46.

Group 3 : PDK/ORP-02

Dendrobium Cluster analyses

Cluster analysis was used to group *Dendrobium* accessions to construct dendrograms based on RAPD markers. The dendrogram separated the 9 accessions into 3 groups (Plate 30).

Group 1 : PDK/ORP-08, PDK/ORP-11, PDK/ORP-13, PDK/ORP-17, PDK/ORP-15, PDK/ORP-16

Group 2 : PDK/ORP-18

Group 3 : PDK/ORP-20

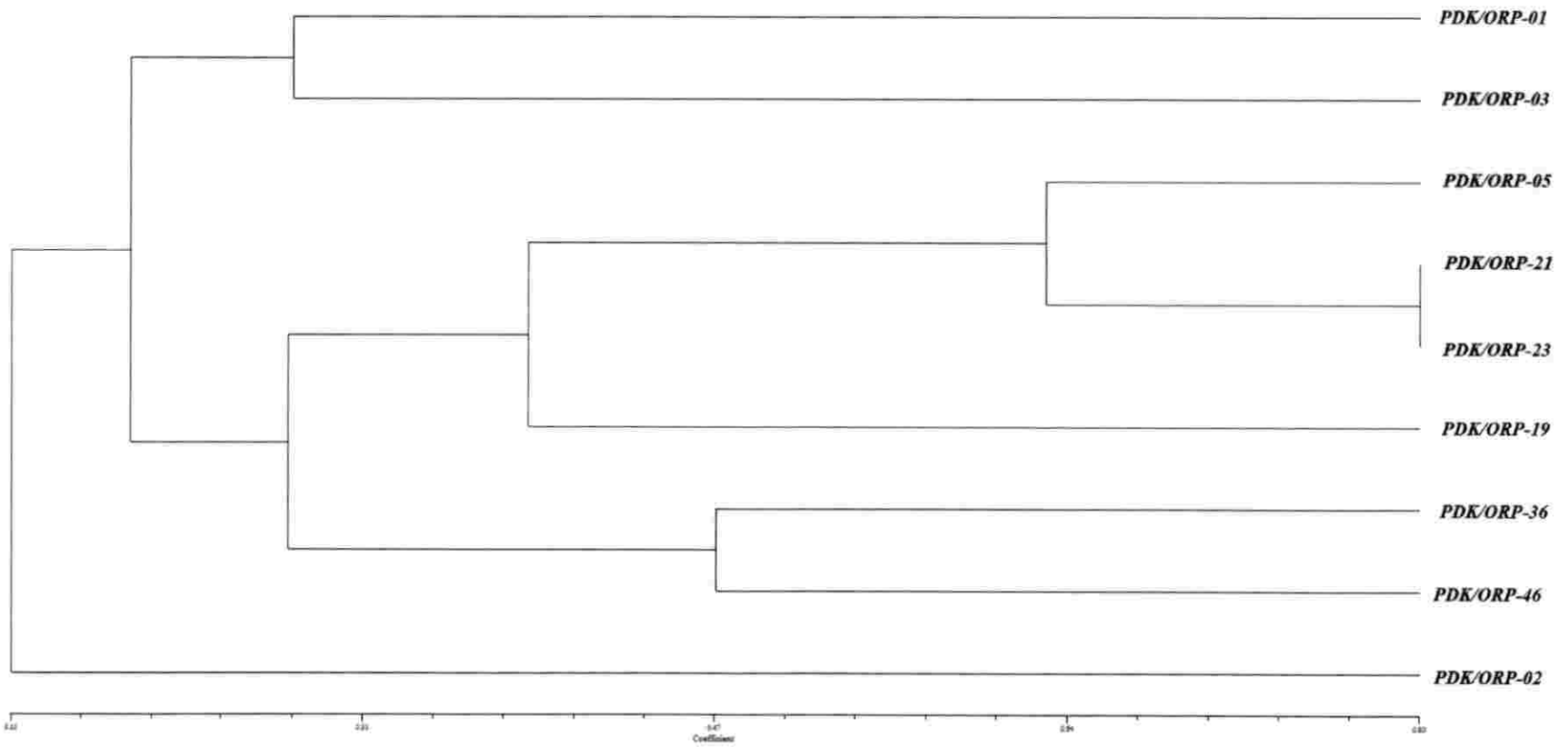


Plate 29: Phylogenetic tree for *Vanda* species based on RAPD markers

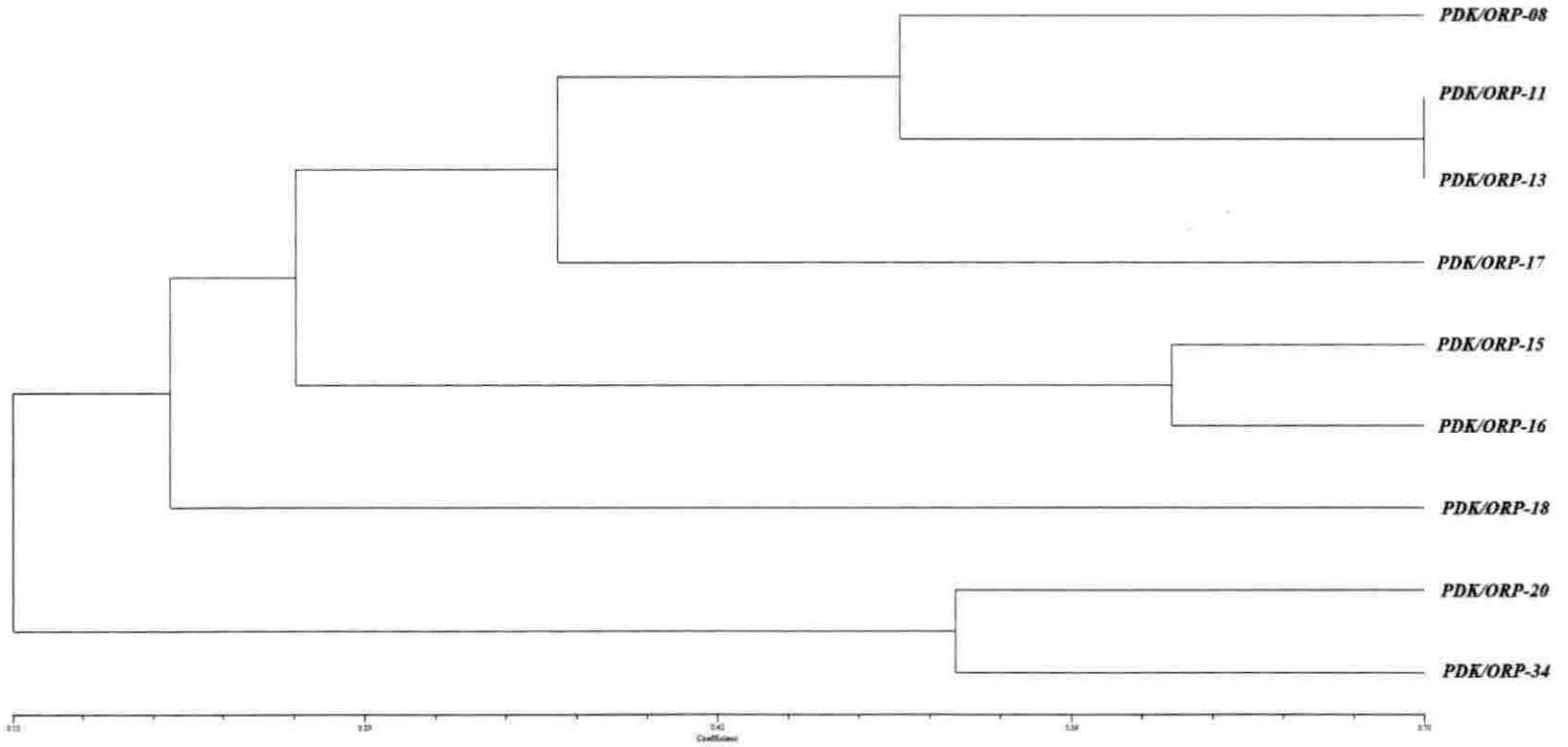


Plate 30: Phylogenetic tree for *Dendrobium* species based on RAPD markers

4.6 MYCORRHIZAL ASSOCIATION

One sample each from the 14 genera was also subjected to investigations for detecting mycorrhizal association (Table 24)

Table 24. Orchid accessions used for the mycorrhizal association studies.

Sl. No.	Accession code	Name of species
1	PDK/ORP-1	*Vanda testacea*
2	PDK/ORP-10	*Phalenopsis hybrid*
3	PDK/ORP-12	*Oncidium tolumina*
4	PDK/ORP-14	Mini *Cattleya*
5	PDK/ORP-18	*Dendrobium hybrid 6*
6	PDK/ORP-22	*Cattleya*
7	PDK/ORP-24	*Cymbidium2*
8	PDK/ORP-28	*Coelogyne breviscapa*
9	PDK/ORP-30	*Aerides maculosa*
10	PDK/ORP-31	*Bulbophyllum fischeri*
11	PDK/ORP-32	*Pholidota imbricata*
12	PDK/ORP-33	*Rhynchostylis retusa*
13	PDK/ORP-39	*Flickingeria nodosa*
14	PDK/ORP-45	*Acampe praemorsa*

The terrestrial and epiphytic species were included for the study in which the epiphytic species were found to be more associated with mycorrhizae, as their roots are in contact with mosses and debris of organic material. The aerial roots and new roots showed infestation only when they had contact with the substrate.

The hyphae and spore of mycorrhizae (Plate 31) detected in all samples were of similar type, but they varied in the colonization percentage (Table 25).

Table 25. Mycorrhizal association of different accessions.

Sl. No.	Genotype	Accession Code	Growth Habit of the orchid	Mycorrhizae		Presence of other endophytes
				Presence	Colonization (%)	
1	*Vanda testacea*	PDK/ORP-1	Epiphytic	*Yes*	65	*Yes*
2	*Phalenopsis hybrid*	PDK/ORP-10	Epiphytic	*No*	-	*Yes*
3	*Oncidium tolumina*	PDK/ORP-12	Terrestrial	*No*	-	*Yes*
4	Mini *Cattleya*	PDK/ORP-14	Epiphytic	*Yes*	60	*No*
5	*Dendrobium hybrid*	PDK/ORP-18	Epiphytic	*No*	-	*No*
6	*Cattleya*	PDK/ORP-22	Epiphytic	*Yes*	70	*No*
7	*Cymbidium2*	PDK/ORP-24	Terrestrial	*Yes*	60	*Yes*
8	*Coelogyne breviscapa*	PDK/ORP-28	Epiphytic	*Yes*	80	*No*
9	*Aeridis maculosa*	PDK/ORP-30	Epiphytic	*Yes*	90	*Yes*
10	*Bulbophyllum fischeri*	PDK/ORP-31	Epiphytic	*Yes*	90	*Yes*
11	*Pholidota imbricata*	PDK/ORP-32	Epiphytic	*Yes*	90	*Yes*
12	*Rhynchostylis retusa*	PDK/ORP-33	Epiphytic	*Yes*	100	*No*
13	*Flickingeria nodosa*	PDK/ORP-39	Epiphytic	*Yes*	72	*Yes*
14	*Acampe praemorsa*	PDK/ORP-45	Epiphytic	*No*	-	*No*

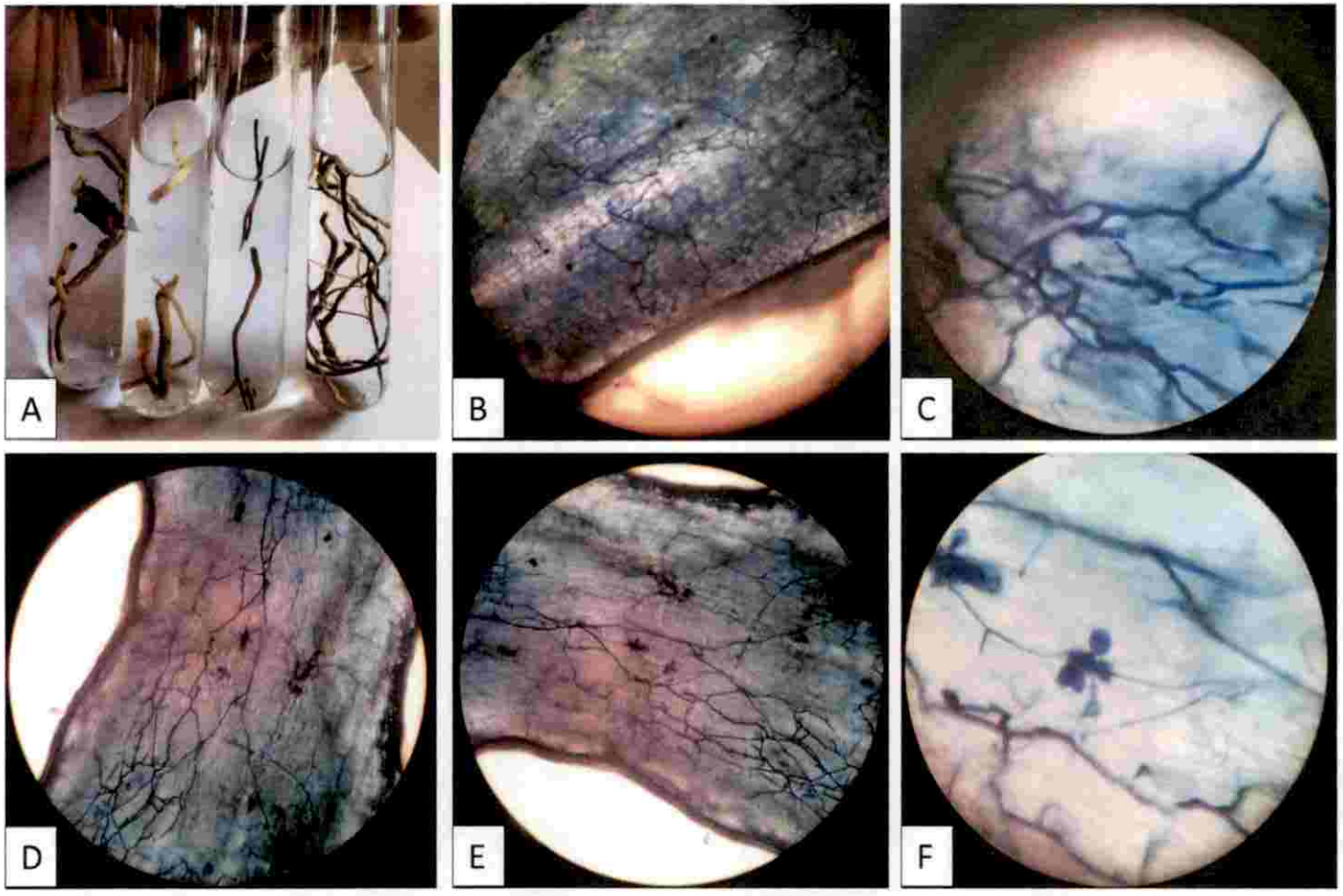


Plate 31: Mycorrhizae colonization in orchid roots (A) root samples, (B) PDK/ORP-30, (C) PDK/ORP-31, (D) PDK/ORP-32, (E) PDK/ORP-33, (F) vesicles.

Discussion

5 DISCUSSION

The results of the present study on the characterization and conservation of orchids specific to the geographical location i.e. Central Western Ghats are discussed in this chapter with their current status and the diversity along with the molecular characterization.

Out of the 1,200 species of orchids in India, 275 species belongs to Western Ghats (Rao, 1998). Sathish Kumar (1986) estimated that there are 85 species of orchids which are endemic to the Western Ghats and 65 species of orchids belonging to Kodagu region (Karnataka) respectively. Central Western Ghats are known for their diversity, among these Brahmagiri Wildlife Sanctuary and Pushpagiri Wildlife Sanctuary are known as treasure troves of orchids. These habitats are rich with various indigenous orchid species that grow epiphytically, terrestrially, or saprophytically in the forests. Its rain forests are also home to some rare species such as *Aerides* sp, *Bulbophyllum* sp.etc.

B. acutiflorum, B. mysorense and B. elangata, Dendrobium Sp., Foxtail orchid *Rhynchostylis retusa Coelogyne* Spp., were found in the high altitudinal shola forest range. These species are endangered and some of them may have not yet been found or discovered. This is mainly because of the loss of habitat resulting from fire, forest damage, illegal logging, and lack of awareness among people about the value of orchids.

The survey included six habitats namely Aralam Wildlife Sanctuary, Ranipuram Wildlife Sanctuary, Pushpagiri Wildlife Sanctuary, Brahmagiri Wildlife Sanctuary, Talakavery Wildlife Sanctuary and Rajeev Gandhi National Park in the Central Western Ghats, well known for their luxuriant vegetation, richness in species. Angiosperm forms the principal group of plant community and some of the habitats have very rare species which are on the verge of becoming extinct. This necessitates the intensive field exploration to locate the species that are presumably in danger of extinction and conservation strategies are to be adopted to protect them.

The preliminary survey indicates that Aralam Wildlife Sanctuary, Ranipuram Wildlife Sanctuary, Pushpagiri Wildlife Sanctuary, Brahmagiri Wildlife Sanctuary, Talakavery Wildlife Sanctuary and Rajeev Gandhi National Park had a good amount of representation of orchids. During the study period, we have assessed total of approximately 9463 accessions of orchids belonging to 30 genera and 70 species. The survey was conducted during 2015-16, and the rescued samples are being maintained in the orchidarium, which were subjected to morphological and molecular characterization.

5.1 DIVERSITY OF ORCHIDS WITHIN DIFFERENT HABITATS

Maximum number of genera was found in the Brahmagiri Wildlife Sanctuary with a total of 23 genera comprising of 39 species, followed by Pushpagiri Wildlife Sanctuary comprising of 34 species under 18 genera as it is said indeed that the Brahmagiri and Pushpagiri Wildlife Sanctuary are treasure troves of orchids. The minimum numbers of genera was noted in Ranipuram Wildlife Sanctuary with a total of 15 genera consisting of 22 species.

Shannon-Wiener index is the popular diversity measure, which is generally based on information theory. The higher value indicates the higher species diversity. Simpson diversity is another diversity measure, which ranged from 0.95112 to almost 0.87597. The lower value indicates the higher species richness.

Shannon index indicated that the highest diversity is recorded at Bharamagiri wild life sanctuary (Shannon index 3.37991) but Simpson index of diversity showed the higher value 0.95834 indicating the lowest species richness. This is followed by Pushpagiri wild life sanctuary with Shannon index 3.274464 and Simpson index of diversity with values 0.95112, Talakavery wild life sanctuary with the Shannon index 3.019068, Simpson index of diversity with values 0.94036 and, Rajeev Gandhi National Park with Shannon index 2.817342 and Simpson index of diversity with values 0.91676 and Aralam Wild life Sanctuary with the Shannon index 2.984732, Simpson index of diversity with values 0.94156. The lowest diversity was observed in Ranipuram Wildlife

Sanctuary compared to all other habitats in the Central Western Ghats with Shannon index 2.540636 and lowest Simpson index of diversity 0.87597 indicating d higher species richness. These two diversity indices reveal that Central Western Ghats have rich orchid diversity.

The main reason for highest diversity in Central Western Ghats is due to the shola type forest and low level of human infiltration into its natural habitat. In general, illegal possession and exploitation of the wild orchids which have high demand in the international markets (Chadha, 1992) and conversion of protected habitats into cultivatable land are main reasons for decrease in the diversity of orchids (Rao, 1977).

5.2 *In situ* conditions (Central Western Ghats)

The observations were recorded on the weather parameter in the 6 natural habitats surveyed, where they are growing under natural conditions. Hodgson *et al*., (1991) has reported that orchids have wide range of habitat as they can grow from sea level up to 4200 m. In the present study, the highest elevation recorded was 1576 m for the accession PDK/ORP-20 which belongs to the *Dendrobium* and found in Brahmagiri Wildlife Sanctuary and the lowest recorded was 14m that which is collected from College of Agriculture, Padannakkad (PDK/ORP). The data obtained shows that the samples are from different climatic regions.

The parameters recorded comprised of the habitat location, latitude, longitude, elevation in meters, light intensity in LUX, temperature at the habitat in degree celsius, canopy temperature in degree celsius, relative humidity in percentage, and wind speed in km/h. The result indicates that the accessions collected are from diverse environments, thus reducing the chance of duplication. This was further confirmed by morphological and molecular characterization.

5.3 Ex situ conditions (College of Agriculture Padannakkad)

The 46 accessions are maintained in an orchidarium at College of Agriculture, Padannakkad, and the weather data were obtained from Department of

Meteorology, Regional Agricultural Research Station (RARS) Pilicode. The maximum temperature was recorded in March to May (33.24-34.41^0 C) and lowest temperatures were recorded in December to January (21.82^0-19.57^0 C). The relative humidity was maximum in July and August (95.35-93.74%) which is the main factor for the luxuriant growth and flowering of orchids (Bose *et al.*, 1999). For most of the orchid species, maximum growth and development observed during the months of in January to March and minimum is recorded in January (56.58%). The wind speed maximum in March month (3.26 km/h) and minimum in August month (0.72 km/h) the daily average maximum rain fall recorded in June (532.60 mm).

5.4 IDENTIFICATION OF ORCHID SPECIES AND ESTABLISHMENT IN ORCHIDARIUM

During survey, a total of 46 accessions consisting of wild types and hybrids were obtained from different places and were maintained in the orchidarium. In general, epiphytic species come up well in tropical warm conditions which were mostly grown in poly houses with additional protection from direct sunlight using green nets (50% shade) which also reduces the inside temperature and increase humidity favoring the luxuriant growth and flowering of orchids. Bose *et al.* (1999) also reported similar results. Out of the of 46 accessions , 41 accessions survived during the later stage of the study and 5 accessions did not get established *viz.*, two *Cymbidium* species (PDK/ORP-26, PDK/ORP-27) from Sikkim, one *Rhynchostylis retusa* (PDK/ORP-37) from Ponnampet and two *Vanda* species (PDK/ORP-40, PDK/ORP-41) from Pushpagiri Wildlife Sanctuary. It may be due to the change in agro-climatic conditions and elevation.

The 46 accessions present in orchidarium comprised of 14 genera in which 26 accessions were native to Central Western Ghats, 4 accessions were from College of Agriculture, Padannakkad, and one species each of *Dendrobium* and *Phalenopsis* tissue cultured plants maintained at Department of Plant Biotechnology. One species each of *Dendrobium* and *Vanda* were collected from

Padannakkad natural habitat, 7 accessions belonging to 3 genera brought from Gangtok, Sikkim (*Vanda, Coelogyne, Cymbidium*) and 9 accessions were hybrids (6 *Dendrobiums,* 1 Mini *Cattleya*, *Oncidium, Phalaenopsis)* brought from Bengaluru.

5.4 Morphological characterization

5.4.1 Vegetative characters

Vegetative characters recorded included qualitative (descriptive) traits, based on Descriptor available from National Research Centre on Orchids, Sikkim and the studies carried for Reproductive Phenology and Morphological Analysis of Indian *Dendrobium* Species by (Lokho 2012). The parameters recorded were nature of pseudo bulb, shape of leaf and leaf apex and quantitative traits such as plant height, leaf length, and width, number of leaves, inter nodal length and number of sprouts.

5.4.1.1 *Vanda spp.*

Out of the 14 genera, *Vanda* had the highest number of accessions (13nos survived out of 15) which varied in their vegetative characteristics like, woody nature of stem, strap leaf shape, channelled, truncate leaf apex, retuse, bilobed and plant height ranged from very small to very large , leaf length short to long , leaf width medium to broad, internodal length short to long and number of leaves varied from few to many. The highest absolute growth was observed in January as the relative humidity was high compared to other months. The species was located at an elevation ranged from 301 m to 1132 m and distributed throughout Central Western Ghats.

5.4.1.2 *Dendrobium spp.*

Dendrobium had the second highest number of accessions after *Vanda* and the vegetative characters varied in the accessions like nature of stem was woody, fleshy, cane fleshy, clavate fleshy, leaf shape was lanceolate, elliptic, leaf apex

was acute, retuse, obtuse and plant height ranged from small to medium, leaf length short to long, leaf width narrow to broad, internodal length small to long and number of leaves varied from few to many. The absolute growth was highest in January as the relative humidity was high compared to other months, The species was located at an elevation ranged from 14 m to 1576 m and distributed through Central Western Ghats.

5.4.1.3 *Cymbidium spp.*

Cymbidium had 4 accessions survived out of six and variations observed in the vegetative characters like, nature of stem was ovoid, conical, leaf shape was linear, linear oblong, leaf apex was acute, bilobed and plant height ranged from small to large, leaf length short to long, leaf width medium to broad, internodal length small to medium and number of leaves varied from few to many. The absolute growth was observed in March to April as compared to other months, The species was located at an elevation ranged from 309 m to 1509 m and distributed in some parts of Central Western Ghats

5.4.1.4 *Bulbophyllum spp.*

Bulbophyllum had 2 accessions belonging to two different species and among the vegetative characters, quantitative traits varied. However, descriptive characters were identical in the two accessions such as nature of stem (bulbous), leaf shape (elliptic), leaf apex (obtuse), plant height (large), leaf length (medium), leaf width (broad), internodal length (small) and number of leaves (few). The absolute growth was highest in April as compared to other months. The species was located at an elevation ranged from 824 m to 1086 m and distributed in all parts of Central Western Ghats.

5.4.1.5 *Coelogyne spp.*

Coelogyne had 2 accessions and the vegetative characters varied, nature of stem fleshy to clavate fleshy; leaf shape oblong, linear oblong; leaf apex acute, and plant height small to large, leaf length short to medium, leaf width medium to

broad, and number of leaves few. The absolute growth was highest in February as compared to other months, The species was located at an elevation ranged from 838 m to 1509 m and distributed in various parts of Central Western Ghats.

5.4.1.6 *Phalenopsis spp.*

Phalenopsis had 2 accessions and the quantitative characters showed variation, whereas descriptive characters were mostly identical, such as fleshy nature of stem, oblong leaf shape, acute leaf apex, and large plant height, leaf length (short to long), broad leaf, small internodal length and number of leaves (few to many). The absolute growth was highest from December to January as compared to other months, The species was located at an elevation ranged from 14 m to 907 m.

5.4.1.7 *Oncidium tolumina*

Oncidium tolumina hybrid was collected from Bengaluru and the vegetative characters were as follows; nature of stem fleshy, leaf shape linear, leaf apex - acute, plant height-small, leaf length-short, leaf width-narrow, and number of leaves few. The absolute growth was observed in December as compared to other months, The species was located at an elevation of 907 m.

5.4.1.8 Mini *Cattleya*

Mini *Cattleya* hybrid was collected from Bengaluru and the vegetative characters were as follows; nature of stem ovoid, leaf shape elliptical, leaf apex obtuse, and plant height small, leaf length short, leaf width narrow, internodal length large and number of leaves a few. There was no change in growth recorded, The species was located at an elevation of 907 m.

5.4.1.9 *Cattleya*

Vegetative characters recorded in *Cattleya* included, cylindrical nature of stem, ligulate leaf shape, bilobed leaf apex, and medium plant height, medium leaf length, leaf width (medium), and number of leaves a few. The absolute growth

was observed in April as compared to other months, The species was located at an elevation of 827 m and distributed in some parts of Central Western Ghats.

5.4.1.10 *Aerides maculosa*

Vegetative characters recorded showed that the nature of stem is woody, leaf shape strap, leaf apex bilobed, plant height large, leaf length short, leaf width broad, and number of leaves few. The absolute growth was maximum in January as compared to other months: The species was located at an elevation of 839 m and distributed in abundant in Central Western Ghats.

5.4.1.11 *Pholidota imbricata*

Vegetative characters recorded in *Pholidota imbricata* included nature of stem bulbous, leaf shape lanceolate, leaf apex acute, and plant height medium, leaf length medium, leaf width broad, internodal length medium and number of leaves many. The absolute growth was maximum in February as compared to other months, The species was located at an elevation of 898 m and distributed in Central Western Ghats.

5.4.1.12 *Rhynchostylis retusa*

vegetative characters recorded in *Rhynchostylis retusa* included nature of stem woody, leaf shape strap, leaf apex a bilobed, plant height large, leaf length medium, leaf width broad, internodal length small and number of leaves many. There was no change in absolute over the 6 months. The species was located at an elevation of 855m and distributed in almost Central Western Ghats.

5.4.1.13 *Flickingeria nodosa*

Vegetative characters recorded in *Flickingeria nodosa* included nature of stem clavate leaf shape s narrow oblong, leaf apex acute, plant height medium, leaf length short, leaf width medium, internodal length small and number of leaves few. The absolute growth was maximum in February as compared to other

months, The species was located at an elevation of 855 m and distributed in Central Western Ghats.

5.4.1.14 *Acampe praemorsa*

Vegetative characters recorded in *Acampe praemorsa* included nature of stem woody, leaf shape strap, leaf apex bilobed, and plant height small, leaf length medium, leaf width broad, internodal length small and number of leaves few. The absolute growth was maximum in February as compared to other months, The species was located at an elevation of 15 m

5.4.2 Reproductive characters

The reproductive characters were recorded based on study carried by Barbhuiya *et al.* (2014). The observations on reproductive characters of 6 accessions which flowered during the period were recorded at flowering phases, viz. *Dendrobium* Hybrid 1, Hybrid 2, Hybrid 3, Hybrid 4, *Dendrobium, and Acampe praemorsa* (PDK/ORP-11, PDK/ORP-13, PDK/ORP-15, PDK/ORP-16, PDK/ORP-20, and PDK/ORP-45 respectively). The flowers were exquisite and showy which exhibited different shapes and colours, Out of the 14 genera, only two reached the flowering stage *viz*., *Dendrobium* (5 accessions) and *Acampe* (one accession viz., *A. praemorsa*, -PDK/ORP-45).

The study revealed that the longest flower duration was for the *Dendrobium* accession (PDK/ORP-20) with 26 days which was collected from the Brahmagiri Wildlife Sanctuary which is a desirable character. Additionally, the flower had bright purple colour with large petals (45.7mm), sepals (35.6mm) and flowered 2 times in interval of 10 months.

Acampe praemorsa flower showed some variations in the colour and pattern of stripes and also it had a pleasant odour to attract the pollinators. The size of flower was small and in cluster and they also developed pods which were cylindrical and long in shape and they were found naturally in almost all the mongo trees in CoA Padannakkad campus (Lokho 2012).

5.4.3 Palynological characters

The Palynological characters were studied for all the 6 accessions. All the *Dendrobium* accessions had 4 pollinia while Acampe *praemorsa* had 2 pollinia and shape also varied which reveal that they are diverse based on studies carried by (Stenzel, 2000) revealed that the Pleurothallidinae as palynologically diverse group.

The present study on vegetative and reproductive characters among the 41 accessions revealed that they were varied in morphological and reproductive characteristics recorded. They were brought from different natural habitats where they have grown under diverse conditions. When brought together and grown under orchidarium, 41 accessions survived and they expressed diverse phenotypic and genotypic characters

5.5 Molecular characterisation

5.5.1 RAPD ANALYSIS

RAPD analysis was done based upon the polymerase chain reaction (PCR) technology. In RAPD, DNA is amplified using random sequences used as primers and the regions of the genome which are complementary are amplified. Scoring is done by comparing the presence or absence of each fragment amplified by the random decamer primer. The analysis will distinguish the similarities or differences between the samples selected. RAPD technique has many advantages such as simplicity and rapidity of the analysis, low cost, availability of a large no of primers and the requirement of the small amount of the DNA for the analysis (Williams *et al*., 1990; Huff *et al*.,1993; Ge *et al* .,1999; Nybom and Bartish, 2000). RAPD technique is more suited to orchids since very little is known about the genetic basis of diversity within the natural population (Xiaohong *et al*., 2007).

The RAPD analysis was done on 17 accessions consisting of 14 genera of orchids which had been rescued from the Central Western Ghats and these 14

genera were screened by 10 RAPD primers which were selected after screening 30 primers. These 10 primers produced a total of 399 scorable DNA fragments of high polymorphism that was present among 14 orchids genera which indicated that these orchid accessions are genetically diverse. RAPD fragments were in the size range of 30 –1700 bp, the number of bands varied from 35 (OPA 20) to 49 (OPE-12) with average of 23.47 bands per assay unit. For separate assay units, PIC values ranged from 0.23 (OPA-18) to 0.34 (OPA-16). The dendrogram based on RAPD markers separated the 17 accessions into 6 groups. The range of similarity coefficients was from 0.08 to 0.48 in 14 genera, and a total of 20 diagnostic bands were detected.OPA 18 produced 6 species specific diagnostic bands (highest number of diagnostic bands) specific to a single accession and this band was not present in other accessions at same base pair level while OPA 16 failed to produce any diagnostic bands.

RAPD experiment on intra generic studies were carried out with 9 accessions each of *Vanda* and *Dendrobium* using 5 primers. The intra generic characterization was carried for detecting polymorphism and duplicates, if any, within each genus. The results obtained based on the analysis of 9 accessions of *Vanda* and *Dendrobium* showed that the accessions in each genus show diversity. The number of bands varied from 27 (OPA 5) to 53 (OPBA-3) with average of 21.33 bands in *Vanda* and 21 (OPA 5) to 61 (OPBA-3) with average of 20.66 bands in *Dendrobium*. A total of 192 RAPD bands in *Vanda* whereas 186 RAPD bands in *Dendrobium*. Amplicons in the size range of 150–1450 bp in Vanda and 175–1625 bp in *Dendrobium* PIC values ranged from 0.23 (OPA-18) to 0.32 (OPBA-3) the range of similarity coefficients for the Vanda was 0.15 to 0.80, and for *Dendrobium* was 0.15 to 0.70 and the dendrogram separated the accessions into 3 groups each.

The results of our study can be used as a baseline for the further diversity assessment and genetic analysis of orchids. RAPD technique could possibly differentiate orchids among and within genera. From the study we can conclude that RAPD can be efficiently used as a tool along with the ecological,

physiological and morphological based classifications. The study revealed that the genomic DNA of orchids provided the phylogenetic information regarding genetic relationship between species.

5.6 MYCORRHIZAL ASSOCIATION STUDIES

Among the accessions of orchids present in the orchidarium at College of Agriculture Padannakkad, a total of 14 accessions belonging to different genera were selected for the mycorrhizal association studies. The terrestrial and epiphytic orchid species had been taken for the study in which the epiphytic species were found to be more associated with mycorrhizae which had mosses and debris of organic material in contact with the roots. The aerial roots and new roots showed infestation only when they had contact with the substrate and the roots which were not in contact were free from the colonization. The hyphae and spore of mycorrhizae were similar in all the samples but they varied in the colonization percentage (60-100%) The 14 genera when examined for the mycorrhizae colonization we could find some endophytic fungi also (Rasmussen 2002; Rasmussen and Whigham 2002; Bidartondo et al. 2004).

Summary

6. SUMMARY

The study on "Characterisation and conservation of promising genotypes of orchids from Central Western Ghats" was carried out at Central Western Ghats and College of Agriculture, Padannakkad, Kasaragod, Kerala during 2014-2016.

The Salient features of the study are summarised below:

The study was based on the survey carried out in 6 natural habitats of Central Western Ghats namely Aralam Wildlife Sanctuary, Ranipuram Wildlife Sanctuary, Pushpagiri Wildlife Sanctuary, Brahmagiri Wildlife Sanctuary, Talakavery Wildlife Sanctuary, Rajeev Gandhi National Park . The survey revealed that, a total of 9463 accessions of orchids, belonging to 70 species comprised under 30 genera, which gave representative samples present in Central Western Ghats. Among these, maximum number of orchid genera was recorded from the Brahmagiri Wildlife Sanctuary with a total of 2337 accessions which represent 39 species coming under 23 genera. Majority of the accessions surveyed belong to the genus *Oberonia* (412 accessions), of which highest number of accessions were observed in the species *Oberonia denticulate* whereas minimum in the Ranipuram Wildlife Sanctuary with a total of 670 accessions, which represents 22 species coming under 15 genera. Majority of the accessions surveyed belong to the genus *Acampe* (461 accessions), of which highest number of accessions were observed in the species *Acampe praemorsa* (461 accessions) The diversity analysis was made by using Shannon Index (H') and Simpson Diversity Index (D), where highest diversity was recorded for Brahmagiri Wild Life Sanctuary, with the values of H' - 3.37991 and D - 0.95834, higher Simpson Index of diversity indicated lowest species richness, whereas it was recorded lowest for Ranipuram Wildlife Sanctuary with value of H' - 2.540636 and D - 0.87597, compared to all other habitat. The preliminary survey indicates higher diversity of orchids in natural forests, which includes rare endangered species such as *Bulbophyllum mysorense, Aerids cripa, A maculosa, Dendrobium crepidatum, Cymbidium bicolor, Rhyncostylis retusa* etc. With respect to the

species diversity four genera had more number of species compared to other genus, *viz.*, *Dendrobium, Bulbphyllum, Hebanaria* and *Oberonia* and maximum number of orchid species were recorded in the genus *Dendrobium* followed by *Bulbophyllum.*

A total of 46 accession comprised of 14 genera and 46 species were rescued and established in the orchidarium of which 26 were native to Central Western Ghats; 4 accessions from College of Agriculture, Padannakkad of which 2 accessions of tissue cultured plants maintained at Department of Plant Biotechnology (one species each of *Dendrobium* and *Phalenopsis*) and 2 accessions from its natural habitat (one species each of *Dendrobium* and *Vanda*); 7 accessions were from Sikkim (1 *Vanda,* 1 *Coelogyne,* and 5 *Cymbidium*) and 9 accessions were hybrids collected from Bengaluru, comprising of 6 *Dendrobiums,* 1 Mini *Cattleya*, 1 *Oncidium,* and 1 *Phalaenopsis.*

The micro climatic observations were recorded during the survey in the 6 natural habitats where the orchids are growing under natural conditions. The parameters which were recorded comprise of the habitat location, latitude, longitude, elevation, light intensity, temperature at the habitat, canopy temperature, relative humidity, and wind speed. The above parameters for *Ex situ* conditions, the weather data was obtained from Department of Meteorology, (RARS), Pilicode representing the Northern Zone of Kerala.

The vegetative and reproductive characters of orchids were recorded based on Descriptor available from National Research Centre on Orchids, Sikkim, and further classified based on their generic characteristics. The vegetative characters reveal variations among the species which can be differentiated from one another. Stem height ranges from 5.9cm to 126cm; the maximum height and number of leaves were recorded in PDK/ORP-5 (*Vanda*); the maximum leaf length and number of sprouts were recorded in PDK/ORP-25 (*Cymbidium*) and PDK/ORP-25 (*Bulbophyllum fischeri*) respectively. The reproductive characters were recorded at flowering phase for 6 accessions. The flowers were exquisite and

showy which exhibit different shapes and colours, the largest flower was recorded in, PDK/ORP-16 (*Dendrobium* hybrid 4) and the longevity of the flower ranges from 8 to 26 days, which was recorded maximum in PDK/ORP-20 (*Dendrobium*, 26 days) rescued from the Brahmagiri Wildlife Sanctuary.

The molecular characterization was done to study the diversity of the selected 17 accessions belonging to 14 genera. Out of 30 RAPD primers 10 RAPD primers were selected based on the amplification bands. The number of bands varied from 35 (OPA 20) to 49 (OPE-12) with average of 23.47 bands. PIC values ranged from 0.23 (OPA-18) to 0.34 (OPA-16). The present study showed 10 primers produced a total of 399 scorable DNA fragments of high polymorphism that were present among 14 orchids genera, amplicons in the size range of 300 – 1700 bp. The dendrogram separated the 17 accessions into 6 groups; the range of similarity coefficients was from 0.08 to 0.48 in 14 genera. A total of 20 diagnostic bands were observed, in which OPA 18 produced 6 species specific bands whereas OPA 16 failed to produce any diagnostic band.

The intra generic characterization was carried for detecting polymorphism and duplicates, if any. Nine accessions each of genera *Vanda* and *Dendrobium* were separately amplified using 5 selected primers. The results obtained based on the analysis of 9 accessions of *Vanda* and *Dendrobium* each. The number of bands varied from 27 (OPA 5) to 53 (OPBA-3) with average of 21.33 bands in *Vanda* and 21 (OPA 5) to 61 (OPBA-3) with average of 20.66 bands in *Dendrobium*. A total of 192 RAPD bands in *Vanda* whereas 186 RAPD bands in *Dendrobium*. amplicons in the size range of 150–1450 bp in Vanda and 175–1625 bp in *Dendrobium* PIC values ranged from 0.23 (OPA-18) to 0.32 (OPBA-3) the range of similarity coefficients for the Vanda was 0.15 to 0.80, and for *Dendrobium* was 0.15 to 0.70 and the dendrogram separated the accessions into 3 groups each.

From the accessions of orchids present in the orchidarium at College of Agriculture, Padannakkad, a total of 14 accessions belong to different genera were selected for the Mycorrhizal association studies. The terrestrial, epiphytic species

were taken for the study in which, the epiphytic species were more associated with mycorrhizae as they are in contact with mosses and debris of organic material.The colonization percent varied from 60 to 100 percent. The aerial roots and new roots showed infestation only when they had contact with the substrate and roots which were not in contact were free from the colonization. The study also revealed some endophytic fungi in some of the genera which needs further detailed studies to confirm the species.

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CHARACTERISATION AND CONSERVATION OF PROMISING GENOTYPES OF ORCHIDS FROM CENTRAL WESTERN GHATS

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ABSTRACT

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Abstract

Orchids occur mainly in humid tropics and temperate regions of the world. They are known for their long lasting and bewitchingly beautiful flowers. Out of the nearly 30,000 species of orchids in the world, India contributes around 1200 species. In India, the north eastern region accounts for about 800 species, while the Western Ghats has around 275 species of orchids.

Western Ghats is one among the 34 biodiversity hotspots identified in the world. However, due to the continuing loss of habitat, fragmentation, expanding human population and other activities, some of the flora and fauna in this region are included in the highly threatened group, orchids being one among them. Many of the orchids in this region are indiscriminately exploited for the local traditional medicine and for horticulture purpose and thus face the danger of being extinct. Hence the present study was undertaken in order to assess the orchid diversity in selected regions in Central western Ghats and to conserve few genotypes which are threatened.

The study on "Characterisation and conservation of promising genotypes of orchids from Central Western Ghats" was carried out at Central Western Ghats and College of Agriculture, Padanakkad, Kasargod, Kerala during 2014-2016. The study was based on the survey carried out in 6 natural habitats of Central Western Ghats namely Aralam Wildlife Sanctuary, Ranipuram Wildlife Sanctuary, Pushpagiri Wildlife Sanctuary, Brahmagiri Wildlife Sanctuary, Talakavery Wildlife Sanctuary and Rajeev Gandhi National Park. The survey revealed that, a total of 9463 accessions of orchids belonging to 70 species classified under 30 genera were present in these habitats. Among these, maximum number of orchid genera was recorded from the Brahmagiri Wildlife Sanctuary (23 genera, 39 species), whereas minimum in the Ranipuram Wildlife Sanctuary (15 genera, 22 species). The diversity analysis was made by using Shannon Index (H') and Simpson Diversity Index (D). The highest diversity was recorded for Bharamagiri Wild Life Sanctuary, with the values of H' - 3.37991

and D - 0.95834, whereas it was lowest for Ranipuram Wildlife Sanctuary with value of H' - 2.540636 and D -0.87597. The preliminary survey indicates higher diversity of orchids in natural forests, which includes rare, endangered species such as *Bulbophyllum mysorense, Aerids cripa, A maculosa, Dendrobium crepidatum, Cymbidium bicolor, Rhyncostylis retusa* etc. With respect to the species diversity four genera had more number of species compared to other genus, *viz., Dendrobium, Bulbphyllum, Hebanaria* and *Oberonia* and maximum number of orchid species were recorded in the genus *Dendrobium* followed by *Bulbophyllum.*

A total of 46 accessions belonging to14 genera were rescued and established in the orchidarium of which 26 were native to Central Western Ghats; 4 accessions from CoA, Padannakkad in which two accessions were tissue culture plants maintained at Department of Plant Biotechnology (one species each of *Dendrobium* and *Phalenopsis*) and 2 accessions from natural habitat of Padanakkad (one species each of *Dendrobium* and *Acampe*); 7 accessions from Sikkim (1 *Vanda,* 1 *Coelogyne,* and 5 *Cymbidium*) and 9 accessions were hybrids collected from Bengaluru, comprising of 6 *Dendrobiums,* 1 Mini *Cattleya,* 1 *Oncidium,* and 1 *Phalaenopsis.*

The vegetative and reproductive characters of orchids were recorded based on descriptor available from National Research Centre on Orchids, Sikkim, and were further classified based on their generic characteristics. The vegetative characters reveal variations among the species which could effectively differentiate the accessions from one another. The plant height ranged from 5.9 cm to 126 cm. The maximum plant height and number of leaves were recorded in PDK/ORP-5 (*Vanda*), the maximum leaf length in PDK/ORP-25 (*Cymbidium*) and highest number of sprouts in PDK/ORP-25 (*Bulbophyllum fischeri*). The reproductive characters were recorded at flowering phase for 6 accessions. The flowers were exquisite and showy which exhibit different shapes and colours, the largest flower was recorded in PDK/ORP-16 (*Dendrobium* hybrid 4) and the

longevity of the flower ranged from 8 days (PDK/ORP-45: *Acampe*) to 26 days (PDK/ORP-20: *Dendrobium* sp. from Brahmagiri).

The molecular characterization was done to study the diversity of the selected 17 accessions belonging to the 14 genera. Out of 30 RAPD primers 10 RAPD primers were selected based on the quality and intensity of amplification. The 10 selected primers produced a total of 399 scorable DNA fragments of high polymorphism that were present among 14 orchids genera, with amplicons in the size range of 300 – 1700 bp. The dendrogram separated the 17 accessions into 6 groups, the range of similarity coefficients was from 0.08 to 0.48 in 14 genera. A total of 20 diagnostic bands were observed, in which OPA 18 produced 6 species specific bands whereas OPA 16 failed to produce any diagnostic band.

The intra generic characterization was carried for detecting polymorphism and duplicates, if any, in *Vanda* and *Dendrobium*. Nine accessions each of genera *Vanda* and *Dendrobium* were separately amplified using 5 selected primers. A total of 192 RAPD bands were produced in *Vanda* (size range : 150–1450 bp) whereas 186 RAPD bands in *Dendrobium* (size range:175–1625 bp) . The range of similarity coefficients for the Vanda was 0.15 to 0.80, and for *Dendrobium* was 0.15 to 0.70 and the dendrogram separated the accessions into 3 groups each.

The Mycorrhizal association study was carried on 14 genera which were subjected to investigations for detecting mycorrhizal association in accessions. The epiphytic species were found to be more associated with mycorrhizae, as their roots are in contact with mosses and debri of organic material. The aerial roots and new roots showed infestation only when they had contact with the substrate. The hyphae and spore of mycorrhizae detected in all samples were of similar type, but they varied in the colonization percentage (60-100%).

The study revealed that, ideal period for general growth of the plants under Padanakkad condition is January-February while the sprouting was more during June. Genera like *Acampe, Bulbophyllum, Coelogyne, Cymbidium, Dendrobium* etc showed faster growth in the orchidarium compared to the rest.

Six of the accessions flowered in the orchidarium among which one was a wild collection from Brahmagiri. It is a promising genotype which can be used for breeding purposes as it has the longest flower duration (26 days), bright colour, large petals (45.7mm) & sepals (35.6mm) and it flowered two times within 10 months. Morphological and molecular data revealed that there is no duplication in the collection, even within the same genus.

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